ISSN 2074-9414 (Print)
ISSN 2313-1748 (Online)

https://doi.org/10.21603/2074-9414-2023-3-2457
https://elibrary.ru/ZPULFC

Original article
Available online at https://fptt.ru/en

Antagonistic Activity of Extremophilic Bacteria Against Phytopathogens in Agricultural Crops

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Received: 20.03.2023
Revised: 19.04.2023
Accepted: 02.05.2023

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Abstract.
Wheat is a vital agricultural crop whose phytopathogens include fungi of the genera *Fusarium* and *Alternaria*. Synthetic pesticides, which are used to combat them, have a negative impact on the environment. Therefore, there is a need for developing safe and effective biopesticides. We aimed to create a consortium of extremophilic microorganisms isolated from natural sources to protect wheat from the diseases caused by *Alternaria* and *Fusarium* fungi.

Ten isolates of extremophilic microorganisms were tested for their antimicrobial activity against *Escherichia coli* and their antagonistic activity against phytopathogens. Based on the results, we developed microbial consortia and evaluated their effectiveness in protecting wheat from phytopathogens.

Five of the strains under study showed the highest activity, three of which were biocompatible, namely *Leclercia* sp., *Sphingomonas paucimobilis*, and *Lactobacillus plantarum*. Four consortia were created from these microorganisms, of which consortium B (with a 2:1:1 ratio of the strains, respectively) proved the most effective. In particular, it increased the area free from the phytopathogen by 4.2% compared to the average values of its individual microorganisms. Also, the consortium had a phytostimulating effect on wheat seedlings (germination of 73.2–99.6%) and protected the seeds infected with phytopathogens from morphometric changes.

The resulting consortium can be used as a biopesticide since it is highly effective in protecting wheat from *Alternaria* and *Fusarium* pathogens.

Keywords. *Triticum aestivum* L., agricultural productivity, phytopathogens, biopesticides, extremophilic bacteria, consortium of microorganisms, environmental safety

Funding. This study was part of the state assignment entitled “Basic research on the development of biopesticides from extremophilic and endophytic microorganisms to help agricultural crops overcome abiotic and biotic stress in Kemerovo Oblast – Kuzbass” (code FZSR-2023-0003).

For citation: Asyakina LK, Serazetdinova YuR, Frolova AS, Fotina NV, Neverova OA, Petrov AN. Antagonistic Activity of Extremophilic Bacteria Against Phytopathogens in Agricultural Crops. Food Processing: Techniques and Technology. 2023;53(3):565–575. https://doi.org/10.21603/2074-9414-2023-3-2457

https://doi.org/10.21603/2074-9414-2023-3-2457
https://elibrary.ru/ZPULFC

Оригинальная статья
https://fptt.ru

Антагонистическая активность экстремофильных микроорганизмов в отношении фитопатогенов сельскохозяйственных культур

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Поступила в редакцию: 20.03.2023
Принята после рецензирования: 19.04.2023
Принята к публикации: 02.05.2023

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Аннотация.
Пшеница (*Triticum aestivum* L.) – важная сельскохозяйственная культура, фитопатогенами которой являются грибы рода *Fusarium* и *Alternaria*. Для борьбы с ними применяют синтетические пестициды, негативно влияющие на окружающую среду и здоровье человека. Разработка безопасных и эффективных аналогов – биопестицидов – является актуальным вопросом. Цель работы заключалась в разработке консорциума на основе экстремофильных микроорганизмов, выделенных из природных источников, для защиты пшеницы от заболеваний, вызванных грибами рода *Alternaria* и *Fusarium*.
Объектами исследования являлись образцы 10 изолятов экстремофильных микроорганизмов. Биохимическую идентификацию изолятов проводили с использованием автоматического микробиологического анализатора Vitek 2 Compact. Изоляты оценивали по показателям антимикробной активности в отношении *Escherichia coli* и антагонистической активности в отношении фитопатогенов по методу встречных культур. На основании полученных данных конструировали микробные консорциумы и оценивали их эффективность и способность защищать пшеницу от фитопатогенов.
Из 10 исследованных изолятов наибольшую активность проявляли 5 штаммов, 3 из которых являлись биосовместимыми: *Leclercia* sp., *Sphingomonas paucimobilis* и *Lactobacillus plantarum*. На основании данных микроорганизмов составили 4 консорциума. Установлено, что совместное применение микроорганизмов повышает их антогонистическую активность: площадь, не занятая фитопатогеном, увеличивалась на 4,2 % по отношению к среднему значению отдельных микроорганизмов, входящих в состав консорциума. Наиболее эффективным являлся консорциум с соотношением штаммов *Leclercia* sp., *S. paucimobilis* и *L. plantarum* 2:1:1 соответственно. Консорциум оказывал фитостимулирующее действие на проростки пшеницы (всхожесть варьировалась в пределах 73,2–99,6 %) и позволял избежать морфометрических изменений при обработке семян, зараженных фитопатогенами.
Разработанный консорциум обладает высокой эффективностью защиты пшеницы от патогенов рода *Alternaria* и *Fusarium* и может использоваться в качестве пестицида биологической природы.

Ключевые слова. *Triticum aestivum* L., продуктивность сельского хозяйства, фитопатогены, биопестициды, экстремофильные бактерии, консорциум микроорганизмов, экологическая безопасность

Финансирование. Работа была выполнена в рамках государственного задания по теме «Фундаментальные исследования по разработке биопестицидов, состоящих из экстремофильных и эндофитных микроорганизмов, для преодоления абиотического и биотического стресса сельскохозяйственными культурами в условиях Кемеровской области – Кузбасса» (шифр FZSR-2023-0003).

Для цитирования: Антагонистическая активность экстремофильных микроорганизмов в отношении фитопатогенов сельскохозяйственных культур / Л. К. Асякина [и др.] // Техника и технология пищевых производств. 2023. Т. 53. № 3. С. 565–575. (На англ.). https://doi.org/10.21603/2074-9414-2023-3-2457

Introduction

Wheat (*Triticum aestivum* L.) is a vital agricultural crop that makes a significant contribution to the food security. However, its yield and nutritional value are greatly reduced by various diseases caused by phytopathogenic microorganisms [1, 2].

Fusariosis is the most common disease in wheat. It is a pathological condition of cultivated and wild plants caused by microscopic fungi of the genus *Fusarium*. This phytopathogen deforms wheat ears and causes them to prematurely lose pigmentation [3, 4]. The grain shrinks, becomes brittle, and its germination capacity decreases [5]. Moreover, mycotoxins accumulate in the grain, posing a threat to human and animal health [6, 7]. According to literature, mycotoxins reduce the resistance of wheat to other phytopathogens [8].

Alternaria blight is another common wheat disease caused by pathogens of the genus *Alternaria*. These microscopic fungi cause black spots of mycelium to form on the ears, disrupting the crop's normal development [9]. In some cases, *Alternaria* pathogens directly affect the grains, causing their shell to darken. This does not affect their ability to germinate, but increases their allergenicity [10].

Synthetic pesticides are most often used to combat these and other phytopathogens that cause infectious diseases in wheat [11]. However, their use is associated with a number of environmental problems. Pesticides are stable compounds that can persist in the environment for a long time, causing pollution of soils, ground and surface waters, as well as the atmosphere [12–14]. When used for extended periods, they accumulate in agricultural soils, causing qualitative and quantitative changes in the microbiome of the rhizosphere and phylosphere. In particular, they decrease the diversity of bacteria and fungi, as well as affect the nitrogen-fixing and colonizing abilities of rhizobacteria [15, 16]. This has a negative impact on cultivated crops such as wheat. Moreover, of considerable concern is the potential of synthetic pesticides for bioaccumulation. They accumulate in the edible parts of the crops, causing harm to human health [17].

Thus, there is a need for alternative methods that exclude the use of synthetic pesticides and ensure the environmentally safe protection of wheat from phytopathogens. According to literature, such methods involve biological means of protection, for example, biopesticides obtained by microbial synthesis [18]. Microorganisms in such preparations are capable of synthesizing a wide range of secondary metabolites that can control the development of infectious diseases in plants [19].

Biopesticides can be developed from extremophilic microorganisms. Their survival strategies in adverse environmental conditions are due to their unique qualities [20]. For example, some extremophiles are able to secrete antibiotic substances to reduce the number of competing species [21]. However, their antagonistic activity is associated with not only antibiotics, but also certain enzymes. For example, *Pseudomonas* sp. isolated from marine sediments produced chitinase, an enzyme that significantly inhibited the development of phytopathogenic fungi [22]. Thus, high antagonistic activity makes extremophiles effective biocontrol agents.

We aimed to develop a consortium based on extremophilic microorganisms isolated from natural sources to protect *T. aestivum* L. from diseases caused by *Alternaria alternata* (F-525), *Fusarium graminearum*, PH-1 (F-877), *Fusarium graminearum* (F- 892), and *Fusarium sporotrichioides* T11 (F-902).

Study objects and methods

We studied the extremophilic bacteria previously isolated from natural sources [23].

Four of the isolates were identified before, while the remaining six were identified using a Vitek 2 Compact automatic microbiological analyzer (Biomerieux, France). For this, microorganisms were cultivated on Columbian blood agar (Himedia, India) for 48 h at 28°C. The resulting cultures were used to prepare suspensions with a McFarland density of 2.70–3.30 [24].

The antagonistic activity of the strains against bacterial cultures was tested on the model microorganism *Escherichia coli*. For this, isolates were grown in the MPB medium at 28°C for 48 h. Then, 1 mL of the culture liquid was centrifuged at 5000 rpm for 5 min, and the supernatant was removed. *E. coli* were inoculated into Petri dishes with a sterile MPA medium. Then, we cut out wells 6 mm in diameter and filled them with 50 μL of the supernatant. The dishes with the wells were placed in a thermostat and kept for 24 h at 28°C. The results were interpreted by measuring the diameter of inhibition zones [25].

The antagonistic activity of the isolates against the phytopathogenic fungi was assessed by the cross-culture method [26]. For this, we placed daily cultures of the isolates onto one side of Petri dishes with potato-glucose agar (Himedia, India) and agar blocks with the phytopathogenic fungi on the other side. The Petri dishes were kept in a thermostat at 28°C, and the inhibition zones were monitored after 3, 5, and 7 days. The control was the Petri dishes with the phytopathogen without the antagonist culture. Radial growth inhibition was calculated according to the formula as follows:

$$\text{Radial growth inhibition} = \left(1 - \left(\frac{dr}{ds}\right)\right) \times 100 \qquad (1)$$

where *dr* is the diameter of the fungus mycelium in a Petri dish with the antagonist culture, mm; *ds* is the diameter of the fungus mycelium in the control, mm.

To create a consortium, we evaluated the biocompatibility of the most promising strains of microorganisms

by their co-cultivation. For this, pure cultures of the isolates were cultivated in MPB medium at 28°C for 48 h. Then, the culture liquid was centrifuged for 5 min at 5000 rpm. Isolate No. 1 was evenly applied onto a Petri dish with the MPA medium, and the supernatant of isolate No. 2 was added into wells 6 mm in diameter. The cultures were cultivated at 28°C for 24 h, followed by the monitoring of inhibition zones. This method was used for all the isolates [27].

The antagonistic activity of the consortia was assessed as described above.

To measure the consortia's ability to reduce the toxic effects of the phytopathogens on wheat (*Triticum aestivum* L.), the seeds were treated with a mixture of the consortium and the phytopathogen in a ratio of 1:1. Prior to this, the seeds were sterilized with a 5% sodium hypochlorite solution for 10 min, washed 5 times with sterile distilled water, and dried for 2 h in a sterile laminar box (Laminar Systems, Russia).

To infect the seeds, they were treated with a phytopathogen suspension (2.5×10^5) prepared by washing off the mycelium and spores of the fungus grown on slant agar at 28°C for 48 h. The seeds were soaked in the suspension for 2 h and then dried under sterile conditions. A consortium of microorganisms for treating the seeds was prepared in a similar way, with the isolates cultivated at 28°C. After the treatment, the seeds were dried and placed on Petri dishes with moistened filter paper discs (25 seeds per dish). The seeds were incubated in a climate chamber (Binder, Germany) at 25°C and 40% humidity. The control was the seeds that were not treated with the phytopathogens or the consortium [28].

Each experiment was performed in triplicate. Mathematical processing was carried out using the Microsoft Office software package.

Results and discussion

The biochemical identification was carried out for 6 microorganisms (Tables 1 and 2).

We identified isolate No. 1 as *Pantoea* sp. (95% probability), isolate No. 4 as *Leclercia* sp. (88% probability), isolate No. 5 as *Sphingomonas paucimobilis* (87% probability), isolate No. 7 as *Stenotrophomonas maltophilia* (86% probability), isolate No. 9 as *Lactobacillus plantarum* (99% probability), and isolate No. 10 as *Staphylococcus aureus* (85% probability).

In our previous studies, isolate No. 2 was identified as *Klebsiella oxytoca* (98% probability), isolate No. 3 as *Enterobacter aerogenes* (86% probability), isolate No. 6 as *Pseudomonas putida* (87% probability), and isolate No. 8 as *Bacillus megaterium* (88% probability).

Escherichia coli was used as a model microorganism to study the antimicrobial activity of the isolates (Fig. 1). Antimicrobial activity is an ability of microorganisms to produce substances that inhibit the development of other microorganisms. It can be used to prevent the growth

Table 1. Biochemical characteristics of gram-negative microorganisms

Таблица 1. Результаты исследования биохимических особенностей грамотрицательных микроорганизмов

No.	Substrate	Inoculate No.			
		1	4	5	7
1	Ala-Phe-Pro-arylamidase	–	–	–	+
2	Adonitol	–	+	–	–
3	L-pyrrolydonyl arylamidase	–	+	–	–
4	L-Arabitol	–	–	–	–
5	D-Cellobiose	+	+	+	–
6	Beta-galactosidase	+	+	+	–
7	H2S	–	–	–	–
8	Beta-N-acetyl-glucosaminidase	–	+	–	+
9	Glutamyl arylamidase pNA	–	–	–	–
10	D-glucose	+	+	+	–
11	Gamma-glutamyl-transferase	+	–	–	+
12	Fermentation/glucose	+	+	–	–
13	Beta-glucosidase	–	+	+	+
14	D-maltose	+	+	–	–
15	D-mannitol	+	+	+	–
16	D-mannose	+	+	+	–
17	Beta-xylosidase	+	+	+	–
18	Beta-alanine arylamidase pNA	–	–	–	–
19	L-proline arylamidase	–	–	–	+
20	Lipase	–	–	–	+
21	Palatinose	–	–	–	–
22	Tyrosine arylamidase	–	–	+	+
23	Urease	–	–	–	–
24	D-sorbitol	+	–	–	–
25	Saccharose/sucrose	+	+	+	–
26	D-tagatose	–	–	+	–
27	D-trehalose	+	+	+	–
28	Citrate (sodium)	+	–	–	+
29	Malonate	–	–	–	+
30	5-keto-D-gluconate	–	–	–	–
31	L-Lactate alkalinisation	+	+	+	+
32	Alpha-glucosidase	–	–	–	+
33	Succinate alkalinisation	–	–	–	+
34	Beta-N-acetyl-galactosaminidase	–	+	–	–
35	Alpha-galactosidase	–	–	–	–
36	Phosphatase	+	–	–	+
37	Glycine arylamidase	–	–	+	–
38	Ornithine decarboxylase	–	–	–	–
39	Lysine decarboxylase	–	–	–	+
40	L-histidine assimilation	–	–	–	–
41	Coumarate	–	+	+	–
42	Beta-glucoronidase	–	–	–	–
43	O/129 resistance (comp. vibrio)	+	–	–	–
44	Glu-Gly-Arg-arylamidase	–	–	–	+
45	L-malate assimilation	+	–	–	–
46	ELLMAN	+	+	–	–
47	L-Lactate assimilation	–	–	–	–

Table 2. Biochemical characteristics of gram-positive microorganisms

Таблица 2. Результаты исследования биохимических особенностей грамположительных микроорганизмов

No.	Substrate	Inoculate No.	
		9	10
1	D-amygdalin	+	–
2	Phosphatidylinositol phospholipase C	–	–
3	D-xylose	–	+
4	Arginine dihydrolase 1	–	+
5	Beta-galactosidase	–	–
6	Alpha-glucosidase	+	+
7	Ala-Phe-Pro Arylamidase	–	–
8	Cyclodextrin	–	–
9	L-Aspartate arylamidase	–	–
10	Beta galactopyranosidase	+	–
11	Alpha-mannosidase	–	–
12	Phosphatase	–	+
13	Leucine arylamidase	+	–
14	L-Proline arylamidase	–	+
15	Beta glucuronidase	–	–
16	Alpha-galactosidase	–	–
17	L-Pyrrolydonyl-arylamidase	–	–
18	Beta-glucuronidase	–	–
19	Alanine arylamidase	+	–
20	Tyrosine arylamidase	–	+
21	D-sorbitol	+	–
22	Urease	–	+
23	Polymixin b resistance	+	+
24	D-galactose	+	+
25	D-ribose	+	+
26	L-Lactate alkalinization	–	+
27	Lactose	+	+
28	N-Acetyl-D-Glucosamine	+	+
29	D-maltose	+	+
30	Bacitracin resistance	+	+
31	Novobiocin resistance	+	–
32	Growth in 6.5% NaCl	+	–
33	D-mannitol	+	+
34	D-mannose	+	+
35	Methyl-B-D-Glucopyranoside	+	–
36	Pullulan	–	–
37	D-raffinose	+	–
38	O/129 Resistance (comp.vibrio.)	+	+
39	Salicin	+	–
40	Saccharose/sucrose	+	+
41	D-trehalose	+	+
42	Arginine dihydrolase 2	–	–
43	Optochin resistance	+	+

of pathogenic microflora in an area, especially in agriculture to increase the survival rate of plants [29, 30].

According to the results, 5 strains did not show any antimicrobial activity against *E. coli*, namely *K. oxytoca*, *S. paucimobilis*, *S. maltophilia*, *B. megaterium*, and *L. plantarum*. The inhibition zones of the other strains

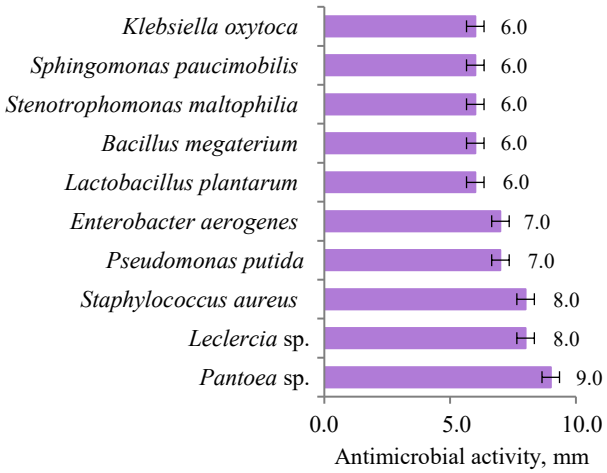


Figure 1. Antimicrobial activity of the isolated microorganisms

Рисунок 1. Результаты антимикробной активности выделенных микроорганизмов

varied from 1.0 to 3.0 mm. Since most of the microorganisms under study did not have bactericidal properties, further tests aimed to measure their antagonistic activity against fungal phytopathogens (Table 3).

As can be seen, the most promising antagonist strains were *E. aerogenes*, *Leclercia* sp., *S. paucimobilis*, *B. megaterium*, and *L. plantarum*. The highest activity against the phytopathogenic fungi *Alternaria alternata* was shown by *Leclercia* sp. (31.3%), *S. paucimobilis* (33.7%), and *L. plantarum* (27.6%) on the 7th day of cultivation. The growth of *Fusarium graminearum* was inhibited by *B. megaterium* (26.8–28.0%) and *E. aerogenes* (31.2–32.3%). The highest antagonistic activity against the genus *Fusarium* (*F. graminearum* and *Fusarium sporotrichioides*) was observed in *Leclercia* sp. (51.0–54.8 and 63.0%, respectively), *S. paucimobilis* (68.4–70.8 and 58.5%, respectively), and *L. lantarum* (80.1–82.4 and 80.3%, respectively). Antagonistic activity against *A. alternata* was absent on the 7th day in *K. oxytoca* (3.5%), *Pantoea* sp. (7.3%), and *S. aureus* (9.4%). No inhibition zone was observed in *S. maltophilia*. The strain *Pantoea* sp. was not resistant to *F. sporotrichioides*. The microorganisms *K. oxytoca* and *S. ltophilia* showed low activity against *F. grainearum* (9.6–10.5%).

Most antagonist strains showed maximum activity against the phytopathogens on the 7th day of cultivation. However, the activity of some strains peaked on the 5th day of cultivation and remained at the same level, e.g., the activity of *Pantoea* sp. and *P. putida* against *A. alternata* (7.3 and 8.2%, respectively), or the antagonicity of *K. oxytoca* against *F. graminearum* PH-1 (F-877) (9.9%).

Our data are consistent with the results reported in modern scientific literature. For example, various

Table 3. Antagonistic activity of the isolated microorganisms against phytopathogenic fungi

Таблица 3. Результаты антагонистической активности выделенных микроорганизмов по отношению к фитопатогенным грибам

Antagonist strain	Incubation time, days	Strain of phytopathogen, %			
		Alternaria alternata (F-525)	*Fusarium graminearum* PH-1 (F-877)	*Fusarium graminearum* (F-892)	*Fusarium sporotrichioides* T11 (F-902)
Pantoea sp.	3	3.8 ± 0.1	10.0 ± 0.5	10.4 ± 0.5	0
	5	7.3 ± 0.2	12.1 ± 0.6	12.6 ± 0.6	0
	7	7.3 ± 0.2	14.6 ± 0.7	15.0 ± 0.8	0
Klebsiella oxytoca	3	0	8.4 ± 0.4	7.2 ± 0.4	11.4 ± 0.4
	5	2.8 ± 0.1	9.9 ± 0.5	9.1 ± 0.5	14.8 ± 0.5
	7	3.5 ± 0.1	9.9 ± 0.5	10.5 ± 0.5	15.3 ± 0.5
Enterobacter aerogenes	3	4.5 ± 0.1	6.4 ± 0.3	10.0 ± 0.5	9.7 ± 0.3
	5	12.6 ± 0.4	18.4 ± 0.9	16.1 ± 0.8	16.2 ± 0.5
	7	16.2 ± 0.5	32.3 ± 1.6	31.2 ± 1.6	21.0 ± 0.6
Leclercia sp.	3	14.2 ± 0.5	30.4 ± 1.5	33.0 ± 1.7	29.7 ± 0.9
	5	18.6 ± 0.6	42.1 ± 2.1	39.6 ± 2.0	43.8 ± 1.3
	7	31.3 ± 1.0	54.8 ± 2.7	51.7 ± 2.6	63.0 ± 1.9
Sphingomonas paucimobilis	3	12.9 ± 0.4	36.0 ± 1.5	35.1 ± 1.1	27.6 ± 1.1
	5	21.5 ± 0.7	44.2 ± 1.9	53.9 ± 1.6	46.3 ± 1.8
	7	33.7 ± 1.1	68.4 ± 2.9	70.8 ± 2.2	58.5 ± 2.3
Pseudomonas putida	3	3.0 ± 0.1	15.7 ± 0.7	14.3 ± 0.4	0
	5	8.2 ± 0.3	17.0 ± 0.7	15.8 ± 0.5	3.8 ± 0.1
	7	8.2 ± 0.3	17.8 ± 0.8	16.1 ± 0.5	6.7 ± 0.2
Stenotrophomonas maltophilia	3	0	0	5.8 ± 0.2	15.0 ± 0.4
	5	0	2.7 ± 0.1	7.4 ± 0.2	17.2 ± 0.4
	7	0	10.2 ± 0.4	9.6 ± 0.3	21.4 ± 0.5
Bacillus megaterium	3	5.2 ± 0.2	7.6 ± 0.4	8.9 ± 0.3	2.5 ± 0.1
	5	8.6 ± 0.3	13.2 ± 0.6	17.3 ± 0.5	11.4 ± 0.4
	7	17.8 ± 0.6	26.8 ± 1.2	28.0 ± 0.9	15.9 ± 0.6
Lactobacillus plantarum	3	12.7 ± 0.4	48.3 ± 2.2	52.1 ± 3.1	50.0 ± 2.8
	5	20.4 ± 0.7	75.1 ± 3.5	74.9 ± 4.5	71.8 ± 4.0
	7	27.6 ± 0.9	82.4 ± 3.8	80.1 ± 4.9	80.3 ± 4.4
Staphylococcus aureus	3	2.8 ± 0.1	11.3 ± 0.5	11.9 ± 0.2	0
	5	5.0 ± 0.2	15.8 ± 0.7	14.7 ± 0.3	6.4 ± 0.2
	7	9.4 ± 0.3	18.1 ± 0.8	19.0 ± 0.4	13.1 ± 0.5

strains of the genus *Bacillus* have been reported to have antagonistic activity against the genus *Alternaria*. Panebianco *et al.* found that the epiphytes *Bacillus cereus* 6C, *Bacillus licheniformis* 4L, *Bacillus thuringiensis* 18D, and *Bacillus velezensis* 23A isolated from PGI Pachino tomatoes inhibited the development of *A. alternata* under the conditions of artificial infection [31]. The L2 strain of *B. megaterium* inhibited the sporulation (by 96.02%) and growth of the mycelium of this phytopathogen [32].

The genus *Pseudomonas* has been reported to suppress *Alternaria*. According to Gupta *et al.*, *Pseudomonas fluorscens* exhibited antimicrobial properties against *Alternaria brassicae* [33]. In addition, the isolate stimulated the growth of agricultural crops.

High antimicrobial activity of *S. maltophilia* against *Alternaria* was observed by Jankiewicz *et al.* [34]. According to the authors, it was due to the release of an active chitinolytic enzyme belonging to the family of 18 glycosyl hydrolases into the substrate. *S. maltophilia* also showed antagonicity against the fungal phytopathogens *Rhizoctonia* and *Fusarium*.

Bacteria of the genus *Pseudomonas* have been reported to exhibit antagonistic activity against phytopathogens of the genus *Fusarium*. For example, Chavéz-Díaz *et al.* described the ability of three *Pseudomonas* isolates from the rhizosphere of Mexican maize to inhibit the growth of the phytopathogen mycelium and increase the rate of seed germination [35]. The seedlings treated with the isolates had a more deveped root system and aerial part. Literature also reports the effective inhibition of *Fusarium* by a strain of *L. plantarum*. This microorganism is able to colonize wheat ears and suppress fungal diseases, increasing the nutritional properties of the grain [36]. *Pantoea* sp. and *Enterobacter* sp. were also found to reduce the impact

of the *Fusarium* phytopathogens on the root system of cultivated plants, both in greenhouse and field conditions [37].

Thus, the microorganisms that we isolated in this study have great potential in the fight against phytopathogens.

To create consortia, we evaluated the biocompatibility of the isolates (Table 4).

We found that the strain *E. aerogenes* was not compatible with *Leclercia* sp., *S. paucimobilis*, and *L. plantarum*, as it suppressed their growth. The strain *Leclercia* sp. had a positive effect on the growth of *S. paucimobilis* and *L. plantarum*. *S. paucimobilis* metabolites inhibited the growth of *E. aerogenes* and *B. megaterium*, while *Leclercia* sp. and *L. plantarum* contributed to their active growth. The microorganism *B. megaterium* was only compatible with *S. paucimobilis*. *L. plantarum* metabolites adversely affected the growth of *E. aerogenes* and *B. megaterium*. Based on the results, we selected those strains that did not exhibit antagonistic activity against each other, namely *Leclercia* sp., *S. paucimobilis*, and *L. plantarum*. We created four variants of consortia based on these strains (Table 5).

The antagonistic activity of the consortia against the phytopathogenic fungi of the genera *Alternaria* and *Fusarium* are shown in Table 6.

As can be seen, consortium B showed high anta gonistic activity against the phytopathogenic fungi. In particular, the area free of *A. alternata* increased by 4.2% in relation to the average value achieved by individual microorganisms in the consortium. Consortium B's activity against the genus *Fusarium* increased by an average of 20.2% on the 7th day of cultivation. Consortium A, however, showed low antagonistic activity on the 7th day against *A. alternata* and *F. graminearum* (F-892), with 9.4 and 5.2% below the average, respectively. Consortium C's activity against *A. alternata* and *F. graminearum* PH-1 (F-877) decreased by 2.2 and 7.6%, respectively. Moreover, its activity against *F. sporotrichioides* was the lowest among the consortia under study, amounting to 48.0% (19.3% lower than the average value). Consortium D showed low antagonistic activity against the fungi of the genus *Fusarium*. In particular, the area free of *F. graminearum* PH-1 (F-877) and *F. graminearum* (F-892) decreased by 16.2 and 14.6%, respectively. All the consortia showed maximum activity against phytopathogens on the 7th day of cultivation.

Table 7 shows the consortia's ability to inhibit the phytopathogenic effect on wheat. When the seeds were treated by both the consortium and the phytopathogens, their germination varied within 73.2–99.6%. The consortia showed the strongest effect against *F. graminearum* PH-1 (F-877).

As can be seen, consortium B had the highest phytostimulating effect, with an average of 24.8 germinated seeds, while consortium A had the lowest phytostimulating effect, with an average of 21 germinated seeds. Consortium B had the greatest effect on wheat seedlings, contributing to a 10.5% higher average coleoptile length than in the control samples. However, when the seeds were inoculated with consortium A, the average coleoptile length was 39.1 mm, i.e., 1.5% shorter than in the control. Treating the seeds with consortium B increased the total length of the seedling roots by 7.2% compared to the control (treated with water). Consortium D, however, decreased this indicator by 1.9% compared to the control, leading to an average length of 185.9 mm. The smallest number of roots per plant was provided by consortia C and D (1.15 and 1.16% below the control, respectively). Consortium B, however, increased the average number of roots 1.13 times compared to the control.

Table 4. Biocompatibility of the isolated microorganisms

Таблица 4. Результаты исследования биосовместимости выделенных микроорганизмов

Strain	*Enterobacter aerogenes*	*Leclercia* sp.	*Sphingomonas paucimobilis*	*Bacillus megaterium*	*Lactobacillus plantarum*
Enterobacter aerogenes		–	–	+	–
Leclercia sp.	–		+	–	+
Sphingomonas paucimobilis	–	+		–	+
Bacillus megaterium	–	–	+		–
Lactobacillus plantarum	–	+	+	–	

Table 5. Composition of consortia

Таблица 5. Состав консорциумов микроорганизмов

Consortium	Composition of consortium
Consortium A	*Leclercia* sp., *Sphingomonas paucimobilis*, *Lactobacillus plantarum* in a ratio of 1:1:1
Consortium B	*Leclercia* sp., *Sphingomonas paucimobilis*, *Lactobacillus plantarum* in a ratio of 2:1:1
Consortium C	*Leclercia* sp., *Sphingomonas paucimobilis*, *Lactobacillus plantarum* in a ratio of 1:2:1
Consortium D	*Leclercia* sp., *Sphingomonas paucimobilis*, *Lactobacillus plantarum* in a ratio of 1:1:2

Table 6. Antagonistic activity of the isolated microorganisms against phytopathogenic fungi

Таблица 6. Результаты антагонистической активности выделенных микроорганизмов по отношению к фитопатогенным грибам

Consortium	Day	Strain of phytopathogen, %			
		Alternaria alternata (F-525)	*Fusarium graminearum* PH-1 (F-877)	*Fusarium graminearum* (F-892)	*Fusarium sporotrichioides* T11 (F-902)
Consortium A	3	12.9 ± 0.4	42.2 ± 1.4	42.8 ± 1.4	40.1 ± 1.3
	5	17.0 ± 0.5	60.8 ± 2.0	59.3 ± 2.0	49.1 ± 1.6
	7	21.5 ± 0.7	69.0 ± 2.3	62.3 ± 2.1	70.2 ± 2.3
Consortium B	3	15.2 ± 0.5	50.9 ± 1.5	53.1 ± 1.8	55.3 ± 1.9
	5	26.4 ± 0.8	76.9 ± 2.3	75.6 ± 2.6	73.2 ± 2.5
	7	35.1 ± 1.1	90.2 ± 2.7	87.6 ± 3.0	86.2 ± 3.0
Consortium C	3	13.4 ± 0.4	37.2 ± 1.2	36.5 ± 1.1	34.6 ± 1.0
	5	19.9 ± 0.6	55.4 ± 1.7	50.0 ± 1.5	41.5 ± 1.3
	7	28.7 ± 0.8	60.9 ± 1.9	67.8 ± 2.1	48.0 ± 1.5
Consortium D	3	10.8 ± 0.4	35.0 ± 1.2	32.4 ± 1.0	38.1 ± 1.2
	5	18.3 ± 0.6	48.2 ± 1.7	48.0 ± 1.6	55.7 ± 1.8
	7	31.2 ± 1.0	52.3 ± 1.9	52.9 ± 1.7	60.3 ± 1.9

Table 7. Growth of wheat treated with consortia and phytopathogenic fungi

Таблица 7. Показатели роста пшеницы, обработанной консорциумами и фитопатогенными грибами

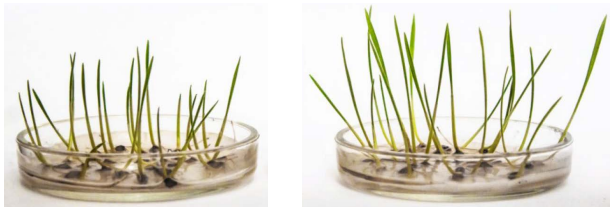
Treatment	Average number of germinated seeds, pcs	Average coleoptile length, mm	Total length of seedling roots, mm	Average number of roots per plant, pcs
Alternaria alternata (F-525)				
Consortium A	19.2 ± 1.4	39.1 ± 1.8	187.4 ± 9.5	3.8 ± 0.3
Consortium B	24.8 ± 1.9	48.3 ± 2.6	190.1 ± 10.2	3.9 ± 0.4
Consortium C	22.0 ± 1.6	40.8 ± 2.1	197.1 ± 10.1	3.5 ± 0.2
Consortium D	24.2 ± 1.5	45.6 ± 2.2	201.3 ± 10.9	3.3 ± 0.2
Fusarium graminearum (F-877) PH-1				
Consortium A	24.2 ± 1.3	46.7 ± 2.3	206.2 ± 10.2	4.1 ± 0.3
Consortium B	24.9 ± 2.0	45.1 ± 2.5	215.6 ± 10.5	4.0 ± 0.2
Consortium C	23.9 ± 1.2	41.2 ± 2.1	186.2 ± 10.3	3.0 ± 0.1
Consortium D	21.4 ± 2.1	43.3 ± 2.2	179.7 ± 9.6	3.4 ± 0.1
Fusarium graminearum (F-892)				
Consortium A	20.1 ± 1.8	36.4 ± 1.9	179.2 ± 9.6	3.9 ± 0.4
Consortium B	24.6 ± 2.3	37.2 ± 2.4	210.6 ± 10.2	4.3 ± 0.5
Consortium C	23.1 ± 1.2	41.3 ± 2.3	201.4 ± 9.5	3.4 ± 0.4
Consortium D	20.2 ± 1.6	38.3 ± 2.1	195.1 ± 10.2	3.2 ± 0.2
Fusarium sporotrichioides (F-902) T11				
Consortium A	20.5 ± 1.6	34.2 ± 1.9	195.7 ± 9.7	4.1 ± 0.3
Consortium B	24.9 ± 2.1	44.9 ± 2.1	200.2 ± 9.4	5.1 ± 0.2
Consortium C	21.8 ± 1.8	40.1 ± 1.9	210.4 ± 10.3	3.3 ± 0.1
Consortium D	18.3 ± 1.7	35.1 ± 1.8	167.3 ± 9.4	3.2 ± 0.1
Control				
Without treatment	24.7 ± 2.1	39.7 ± 2.5	189.4 ± 10.17	3.8 ± 0.1

Figure 2 shows the seedlings treated with consortium B and *F. graminearum* (F-892), as well as the control sample without this treatment.

Noteworthily, we found no visual morphometric defects in any of the wheat samples treated with the consortia. The sprouts had a uniform color that did not differ from that of the control samples (untreated with the consortia and phytopathogens).

Conclusion

We identified six extremophilic microorganisms and studied the antagonistic activity of ten isolates against the model bacterium *Escherichia coli*. The samples showed low bactericidal properties. Next, we studied their activity against phytopathogenic fungi such as *Alternaria alternate* (F-525), *Fusarium graminearum* PH-1 (F-877), *Fusarium graminearum* (F-892), and *Fusarium*



Control
(seed treatment with
water without pathogen)

Seed treatment
with consortium B
and *Fusarium graminearum*
(F-892)

Figure 2. Wheat seedlings treated with consortium B and *Fusarium graminearum* (F-892)

Рисунок 2. Внешний вид проростков пшеницы, обработанных консорциумом В и *Fusarium graminearum* (F-892)

sporotrichioides T11 (F-902). According to the results, the most promising antagonist strains were *Enterobacter aerogenes*, *Leclercia* sp., *Sphingomonas paucimobilis*, *Bacillus megaterium*, and *Lactobacillus plantarum*. We also found that most of the antagonist strains showed maximum activity on the 7th day of cultivation. The isolates were then tested for biocompatibility to form consortia. As a result, we selected those strains that did not exhibit antagonistic properties against each other, namely *Leclercia* sp., *S. paucimobilis*, and *L. plantarum* in the ratios of 1:1:1, 2:1:1, 1:2:1, and 1:1:2 (consortia A, B, C, and D, respectively). The consortia were tested for antagonistic activity against the phytopathogenic fungi. We found that consortium B, which consisted of *Leclercia* sp., *S. paucimobilis*, and *L. plantarum* in a ratio of 2:1:1, increased the antagonistic properties of its individual microorganisms. Also, this consortium had a phytostimulating effect on wheat seeds, increasing the average coleoptile length by 10.5% compared to the control and contributing to an average germination rate of 24.8 seeds. In addition, the joint treatment of seeds with the consortium and the phytopathogens did not cause any visual morphometric defects in wheat. Thus, this consortium proved highly effective in protecting wheat from Alternaria and Fusarium pathogens.

Contribution

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

Conflict of interest

The authors declare no conflict of interest regarding this publication.

Критерии авторства

Авторы в равной степени участвовали в написании рукописи и несут равную ответственность за плагиат.

Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов в данной публикации.

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