

The biopotential of extremophilic microorganisms isolated from Kuzbass for protection and growth stimulation of oat (*Avena sativa* L.)

Yuliya Serazetdinova^{1,*}, Ekaterina Borodina¹, Daria Kolpakova¹, Anna Frolova¹, Natalya Fotina¹, Sergey Tikhonov², and Lyudmila Asyakina¹

¹Kemerovo State University, Kemerovo, Russia

²Ural State University of Economics, Yekaterinburg, Russia

Abstract. Biological plant protection products are extensively utilized in agriculture owing to their safety, efficacy, and environmental friendliness. The utilization of these funds in locales with challenging weather patterns is constrained. In order to ensure the successful use of biological plant protection products, it is rational to search for microorganisms adapted to the specific soil and climatic conditions of the territory. The objective of the investigation is to evaluate the antagonistic and growth-stimulating properties of the consortium of extremophilic microorganisms of Kuzbass, and to conduct laboratory testing on oats (*Avena sativa* L.). The work used microorganisms isolated at the initial stages of the research, such as *Leclercia* sp., *Sphingomonas paucimobilis*, and *Lactobacillus plantarum*. The findings revealed that these microorganisms are capable of producing gibberellic acid, dissolving phosphates, and retaining atmospheric nitrogen. Furthermore, the co-cultivation of microorganisms facilitates the enhancement of these indicators. The negative impact of phytopathogens (*Alternaria alternata* (F-525), *Fusarium graminearum* (F-877) PH-1, *Fusarium graminearum* (F-892) and *Fusarium sporotrichioides* (F-902)) on oats in laboratory conditions was reduced by a consortium based on these microorganisms. Therefore, the consortium has the potential to develop commercial drugs. Field experiments are planned in the future.

1 Introduction

Bio-based plant protection substances are widely employed in environmentally friendly agricultural practices [1]. Biopesticides are distinguished from pesticide chemicals by their safety for the environment, as well as for humans and animals. The utilization of biological plant protection agents not only prevents the emergence of pathogen resistance, but also mitigates the potential for the accumulation of toxic substances in agricultural products [2].

Biological preparations are widely used to protect various crops, especially cereals, which are increasingly threatened by toxinogenic fungi. Every year, phytopathogenic fungi of the

* Corresponding author: serazetdinova2000@mail.ru

genus *Fusarium*, *Alternaria*, *Pyricularia*, and *Aspergillus* cause significant production losses (more than 40% of the cereal yield) [3]. In this context, the primary focus in the development of biopesticides should be devoted to the exploration of promising microbe-plant interactions that enhance the resistance of cereal crops to fungal illnesses.

Despite the numerous advantages of biological plant protection products, there are also certain challenges associated with their application. Biopesticides are living systems, therefore environmental stress factors have the potential to significantly diminish their efficacy or result in their complete inactivation [4]. The issue of the Kemerovo region – Kuzbass is of significant concern, as it is situated in a region that is susceptible to risky agricultural practices [5]. Weather conditions that make cereal crops less productive make them more vulnerable to phytopathogens. Nonetheless, biopesticides that possess the capability to safeguard plants under such arduous circumstances are currently not available on the market [6].

The resolution of issues arising from unfavorable environmental conditions can be achieved through the utilization of extremophilic microorganisms. They have been adapted to adverse environmental factors, resulting in their efficacy being maintained in field conditions. Furthermore, survival strategies of extremophiles are determined by the presence of unique qualities that are not found or less pronounced in other microorganisms [7]. Some of them are reported to be capable of secreting antibiotic substances to reduce the number of competing species [8]. It is important to note that the antagonistic activity of extremophilic microorganisms is not only associated with the synthesis of antibiotics, but also with the presence of certain enzymes, such as chitinases and β -1,3-glucanases. They are capable of destroying the cell walls of phytopathogenic fungi and are considered the most important metabolites in biological control. There is research showing that some extremophilic microorganisms are capable of producing chitinases and β -1,3-glucanases [9]. According to a research by S. Abdelaziz et al., the extremophiles *Bacillus halotolerans*, *Massilia alkalitolerans*, and *Bacillus aryabhatai* produced lytic enzymes and siderophores. Furthermore, the strains displayed high antagonistic activity against plant phytopathogens as *Pythium ultimum* and *Rhizoctonia solani* [10]. Extremophilic microorganisms have been reported to express plant defense mechanisms through the synthesis of xylanase and cellulase enzymes [11].

The objective of the investigation is to evaluate the antagonistic and growth-stimulating properties of the consortium of extremophilic microorganisms of Kuzbass, and to conduct laboratory testing on oats (*Avena sativa* L.).

2 Materials and Methods

The subject matter of the investigation comprised extremophile microorganisms *Leclercia* sp., *Sphingomonas paucimobilis*, and *Lactobacillus plantarum*, which were isolated from natural sources during the initial stage of the research [12], as well as a consortium based on them in a ratio of 2:1:1 [13].

The ability to synthesize gibberellic acid (GA) was assessed by preparing a suspension of the test strains in 1 mL of Luria-Bertani medium in the Miller modification (MB) to a turbidity coefficient of 0.8–1.0 according to McFarland (titer 1.5×10^8 CFU/mL) using a Densichek plus densitometer (Sendle, Russia). The resulting suspension was then added to 10 mL of LB medium. Suspension were cultured on an LSI-3016A/LSI-3016R incubator shaker (Daihan Labtech, South Korea) for 72 hours at a temperature of 28 ± 2 °C and 100 rpm.

The culture liquid was centrifuged at 8000 rpm for 25 minutes, and 560 μ L of a 10.6% solution of potassium ferrocyanide III and 1 M zinc acetate were added to 4 mL of the resulting supernatant. The resulting mixture was centrifuged for a duration of 10 minutes at a rotational speed of 4500 rpm, and subsequently, 2 mL of 30% hydrochloric acid was incorporated into the resulting supernatant. The solution was kept at room temperature for 75 minutes, and the optical density was measured using a UV 1800 spectrophotometer

(Shimadzu, Japan) at a wavelength of 254 nm. A nutrient medium containing 5% hydrochloric acid in a 1:1 ratio was utilized as a reference solution. The amount of synthesized gibberellic acid was determined according to the calibration graph of a standard GA solution ranging from 20 to 400 µg/mL [14].

The ability to solubilize phosphates was determined according to the method of M.R. Swain et al. [15]. In order to quantify the solubilization of phosphates, a medium of the following composition was employed, g/L: glucose 10.0, calcium phosphate 5.0, magnesium chloride 5.0, magnesium sulfate 0.5, and ammonium sulfate 0.1. A suspension of microorganisms with a turbidity coefficient of 0.8–1.0 according to McFarland was added to the nutrient medium and incubated for 48 hours. The amount of available phosphate was assessed spectrophotometrically using the method Sandilya et al. [16]. The quantity of phosphorus that is readily available was determined through calibration against a KH₂PO₄ standard curve.

For determination of the ability to fix atmospheric nitrogen, 1 mL of the previously obtained suspension was added to 10 mL of nitrogen-free medium. The mediums composition is similar to that used for isolation [17]. The obtained mixture had been cultivated for 96 hours at a temperature of 28–30 °C. Then, the cultural liquid was separated from the cells using the previously described technique. An analysis of the nitrogen content in the cultural liquid was performed using the Rapid N Cube analyzer (Elementar, Germany).

In order to assess the ability of consortia to reduce the toxic effect of phytopathogens on oats (*Avena sativa* L.), seeds were treated with a mixture of consortium and phytopathogen in a 1:1 ratio. To accomplish this, the seeds were sterilized in a 10% sodium hypochlorite solution for 10 minutes, washed five times with sterile distilled water, and dried for two hours under sterile conditions in a laminar flow hood (Laminar Systems, Russia).

Seeds were infected by treatment with a suspension of the phytopathogen (prepared by washing off the mycelium and spores of the fungus grown on slanted agar at a temperature of 27±2 °C for 72 hours). The concentration of fungal phytopathogens in the suspension was at least 2.5×10⁵ CFU/mL. The seeds were submerged in the suspension for two hours before being dried in sterile conditions. The consortium of microorganisms for seed treatment was prepared in a similar manner, wherein isolates were grown at a temperature of 28±2 °C for a duration of 48 hours. The seeds were dried after treatment and placed on Petri dishes with moistened filter paper discs (20 seeds per dish). The seeds were incubated in a climate chamber (Binder, Germany) at a temperature of 25 °C and a humidity of 40–50%. Seeds that were not subjected to treatment with phytopathogens and the consortium, as well as those that were treated solely with the consortium, were utilized as a control [18]. On the 10th day, an assessment of germination, number of roots, and measurement of root and sprout length was carried out.

Each experiment was replicated three times. The mathematical processing was performed using the Microsoft Office software package. The obtained data were analyzed using a one-time paired Students test for each pair of interests. Differences were considered to be statistically significant at p<0.05.

3 Results

The results of the determination of the ability to produce gibberellic acid, phosphate solubilization, and nitrogen fixation are presented in Table 1.

Table 1. The characteristics of microorganisms and consortium

Sample	GA, µg/mL	Phosphate solubilization, mg/mL	Nitrogen fixation, mg/mL
<i>Leclercia</i> sp.	10.29±0.29	30.21±0.96	9.72±0.29

<i>Sphingomonas paucimobilis</i>	2.61±0.08	42.26±1.20	0.05±0.01
<i>Lactobacillus plantarum</i>	16.92±0.53	23.05±0.63	2.55±0.06
Consortium	23.64±0.65	52.22±1.71	14.33±0.48

According to research, the synthesis of gibberellic acid in strains ranges from 2.61 to 16.92 µg/mL, the content of soluble phosphorus is between 23.05 and 42.26 mg/mL, and the content of nitrogen is between 0.05 and 9.72 mg/mL. A consortium based on *Leclercia* sp., *Sphingomonas paucimobilis*, and *Lactobacillus plantarum* in a 2:1:1 ratio showed better performance in the studied characteristics than individual strains. Therefore, the consortium synthesized 2.38 times more gibberellic acid, solubilized 1.64 times more phosphates, and fixed 3.49 times more nitrogen compared to the average value by microorganisms.

There are results of the consortiums ability to inhibit phytopathogenic effects on oats presented in Table 2. Samples 1, 3, 5, 7, and 8 were treated with a phytopathogen. Samples 2, 4, 6, and 8 were treated with the consortium and the phytopathogen.

Table 2. The oat growth indicators are on day 10

Treatment Option	Germination rate, %	Average root length, mm	Average sprout length, mm	Average number of roots per plant, pcs.
<i>Alternaria alternata</i> (F-525)				
Sample 1	62.3±0.6	73.8±0.7	107.1±0.9	2.9±0.2
Sample 2	72.9±0.7	76.9±0.7	118.6±1.1	4.1±0.3
<i>Fusarium graminearum</i> (F-877) PH-1				
Sample 3	69.1±0.7	75.4±0.7	108.3±0.9	2.8±0.2
Sample 4	74.0±0.7	78.2±0.8	120.3±1.1	4.0±0.3
<i>Fusarium graminearum</i> (F-892)				
Sample 5	80.4±0.6	72.3±0.7	113.1±1.1	3.2±0.2
Sample 6	89.9±0.7	84.5±0.8	141.7±1.3	4.4±0.3
<i>Fusarium sporotrichioides</i> (F-902) T11				
Sample 7	73.9±0.7	76.8±0.7	110.5±1.0	3.0±0.2
Sample 8	90.1±0.8	80.8±0.8	121.6±1.2	4.1±0.3
Контроль				
Consortium	95.6±0.9	87.5±0.9	146.8±1.5	4.7±0.3
No treatment	88.7±0.7	78.9±0.8	138.4±1.4	3.5±0.3

The treatment of oat seeds with a consortium and phytopathogenic fungi *Fusarium graminearum* (F-892) and *Fusarium sporotrichioides* (F-902) T11 resulted in a significant germination rate of 90%, which is 1.3% higher than the untreated control. The lowest rate of seed germination was observed in samples 1 and 3 – an average of 65.7%, which is 23% lower than the untreated control. The average length of the roots increased by 5.5% when treated with the consortium and phytopathogens, compared to the same treatment with phytopathogens alone. The average length of the shortest root was 72.3 mm when treated with *Fusarium graminearum* (F-892), which is 6.6% lower than the untreated control. Treatment with phytopathogens resulted in a decrease in average shoot length, which was 15.8% lower than with the consortium and phytopathogens, and 28.7% lower than with the untreated control. The number of lateral roots per plant decreased by 0.5 when treated with phytopathogens alone compared to the untreated control, and by 1.2 more when treated with phytopathogens and the consortium compared to treatment with phytopathogens alone.

4 Discussion

The results of investigations have revealed that the production of gibberellic acid ranges from 2.61 to 16.92 $\mu\text{g/mL}$ in various strains. The proportion of soluble phosphorus varies from 23.05 to 42.26 mg/mL , while that of nitrogen varies from 0.01 to 9.72 mg/mL . The obtained results are in agreement with the literature data. According to the research conducted by W. Chen et al. [19], the strain *Leclercia adecarboxylata* QDSM01 possesses the capability to solubilize phosphates (with a soluble phosphorus content of 26.86 $\mu\text{g/mL}$) and fix nitrogen (with a nitrogen content of 13.7 mg/mL). Nonetheless, there is a dearth of data regarding the production of GA by *Leclercia*. S. Yang et al. [20] reported that *Sphingomonas paucimobilis* ZJSH1 elevates the nitrogen concentration in the medium by 1.15 mg/L . V. Martinez-Gallegos et al. [21] conducted a study to examine the capacity of *Sphingomonas paucimobilis* pshba to produce GA (1.7 mg/mL of nutrient medium) and to solubilize phosphates (39.5 g/mL). The literature sources contain insufficient information regarding the synthesis of GA, the capacity to solubilize phosphates, and nitrogen fixation by *Lactobacillus plantarum*. A consortium comprising *Leclercia* sp., *Sphingomonas paucimobilis*, and *Lactobacillus plantarum* in a ratio of 2:1:1 demonstrated superior characteristics in terms of the studied parameters in comparison to the individual strains.

As per the obtained findings, it has been determined that the collective of microorganisms exhibits a phytostimulating effect on the seeds of oats (*Avena sativa* L.) and mitigates biotic stress resulting from adverse effects of phytopathogens. The evidence from the published sources suggests that the microorganisms investigated in this study are capable of reducing both biological and environmental stresses. In the research conducted by A. Bilal et al., it was discovered that the strain *Leclercia adecarboxylata* PAB19 has the potential to alleviate water stress by enhancing its biological and biochemical attributes [22]. In the work of S.-M. Kang et al., the strain *Leclercia adecarboxylata* MO1 was found to promote plant growth under control conditions as well as under the toxic effects of Zn (2 and 5 mM). Furthermore, *L. adecarboxylata* exerts a positive influence on physicochemical properties by reducing the absorption of hydrogen peroxide and Zn by roots and shoots, thereby enhancing antioxidant systems [23]. The strain *Sphingomonas sediminicola* DAE20, as determined by C Mazoyon and colleagues, affects the plants root system, promoting branching, by releasing auxin in the form of indole-3-acetic acid [24]. The researchers B. Turk et al. assessed the impact of a mixture of *Lactobacillus casei*, *Lactobacillus plantarum*, *Rhodopseudomonas palustris*, and *Saccharomyces cerevisiae* on plant growth, yield, and certain qualitative indicators. The findings revealed that the administration of microorganisms aids plant development and enhances their weight [25].

5 Conclusion

During the present investigation, the growth-stimulating characteristics of extremophilic microorganisms, namely *Leclercia* sp., *Sphingomonas paucimobilis*, and *Lactobacillus plantarum*, which were isolated from natural sources in the Kuzbass region, were evaluated. Microorganisms possess the capability to synthesize growth promoters, solubilize phosphates, and fix atmospheric nitrogen. Moreover, the cultivation of these strains in co-culture facilitates the attainment of synergistic effects and the enhancement of these promising attributes. The microbial consortium successfully mitigated the adverse effects of biotic stress induced by *Alternaria alternata* (F-525), *Fusarium graminearum* (F-877) PH-1, *Fusarium graminearum* (F-892), and *Fusarium sporotrichioides* (F-902) T11, in laboratory experiments. It is therefore possible to use the constructed consortium as a complex biopesticide. Future research will examine the effectiveness of the consortium

in field experiments. The outcome will entail the creation of a commercial product aimed at promoting sustainable agricultural practices in the Kuzbass region.

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