Foods and Raw Materials. 2024;12(2)

ISSN 2308-4057 (Print)
ISSN 2310-9599 (Online)

Research Article
Open Access

Available online at http://jfrm.ru/en
https://doi.org/10.21603/2308-4057-2024-2-613
https://elibrary.ru/NAHYDP

Extremophilic bacteria as biofertilizer for agricultural wheat

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Received 18.07.2023; Revised 20.10.2023; Accepted 07.11.2023; Published online 27.12.2023

Abstract:
Wheat (*Triticum L.*) is a strategically important agricultural crop because its quality and yield provide food security for the population. Biological fertilizers improve the growth and development of agricultural crops. Unlike chemical ones, they have no toxic effect on people and the environment. This research assessed the positive effect of extremophilic microorganisms isolated from coal dump soils of the Kemerovo Region (Russia) on the growth and development of wheat.

The study featured bacterial isolates of *Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter*, as well as their consortia in four different ratios: 1:1:1 (Consortium A), 2:1:1 (Consortium B), 1:2:1 (Consortium C), 1:1:2 (Consortium D), respectively. The beneficial effect was assessed by determining such factors as nitrogen fixation, solubilization of phosphates, potassium, and zinc, and production of gibberellic acid, siderophores, and hydrogen cyanide. The wheat samples were checked for germination, root length, and stem length.

R. radiobacter demonstrated the best nitrogen fixation properties. Consortium D, with two shares of *R. radiobacter*, yielded the best results for zinc solubilization. *R. radiobacter* proved to be the most efficient potassium solubilizer while the isolate of *A. denitrificans* was the best phosphate solubilizer. The largest amount of gibberellic acid belonged to *K. oxytoca*. Consortium C, which included two shares of this isolate, appeared to be the most effective siderophore producer. All samples but *A. denitrificans* were able to produce hydrogen cyanide. The best seed germination rate (84%) belonged to Consortium C, which contained a double share of *K. oxytoca*. Consortia C and B (two shares of *A. denitrificans*) had the greatest positive effect on the root length. Treatment with Consortium B resulted in the longest average stem length.

Extremophilic microorganisms isolated from coal dump soils of the Kemerovo Region (Russia) had a good potential as biofertilizers that could improve wheat quality and local food security.

Keywords: Food safety, wheat, biofertilizers, extremophilic microorganisms, seed germination

Funding: The research was part of state assignment FZSR-2023-0003: Biopesticides based on extremophilic and endophytic microorganisms as a means to overcome abiotic and biotic stress in agricultural crops in the Kemerovo Region (Kuzbass).

Please cite this article in press as: Faskhutdinova ER, Fotina NV, Neverova OA, Golubtsova YuV, Mudgal G, Asyakina LK, *et al.* Extremophilic bacteria as biofertilizer for agricultural wheat. Foods and Raw Materials. 2024;12(2):348–360. https://doi.org/10.21603/2308-4057-2024-2-613

INTRODUCTION

The current world population of 7.9 billion people is expected to reach 10 billion people by 2050 [1]. Such rapid growth rate challenges the agricultural sector as the demand for food resources keeps growing. According to the Food and Agriculture Organization of the United Nations, the global production will have to increase by 60% over the next two decades to feed the growing population [2]. Almost 90% of all food comes from 12 crops and 14 animal species [3]. In particular, wheat, rice, and corn cover more than half of the world's food demand.

The agricultural importance of wheat can hardly be overestimated. Wheat is the main source of plant proteins in human and animal diets in more than 80 countries [4, 5]. In fact, one third of global population obtain 13–57% of their caloric intake from wheat, which makes wheat their main source of energy. Wheat is the second main source of energy in 26 countries, including China and India, and the third main source of energy in

another 16 countries. In total, about 85% of the world's population depend on wheat as their main source of energy. Therefore, increasing wheat production volumes is one of the most urgent tasks that the food industry has to tackle in the nearest future.

Traditionally, crop farming relies on chemical methods, which means severe man-induced environmental load and poor phytosanitary condition of agricultural lands [6–8]. Bacterial plant growth promotion, known in Russian agriculture as biologization, is a promising direction in wheat cultivation as it harnesses the potential of plant growth-stimulating bacteria [9–11]. These bacteria and their metabolites provide biofertilizers that boost the rhizospheric biogenicity, thus improving the ecological condition of the entire agrocenosis. Under proper conditions, microorganisms produce metabolites of agricultural importance [12]. Microorganisms and their metabolites break down complex soil minerals, turning them into growth-promoters for a particular crop.

Nitrogen is vital for plant growth. Soil contains two main forms of nitrogen, i.e., inorganic, or mineral, nitrogen (2%) and organic nitrogen (98%) [13–16]. Inorganic nitrogen includes ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), and nitrates (NO_3^-) [17]. Organic nitrogen is to be found in organic nature, e.g., soil biota, fresh remains of animals and plants, etc., as well as in inorganic nature, e.g., as humified or non-humified compounds [18]. Mineral nitrogen is available to plants either as ammonium nitrogen (NH_4^+-N) or as nitrate nitrogen (NO_3-N) [19]. Organic nitrogen becomes available to plants only after mineralization into ammonium or nitrate [20]. Biological nitrogen fixation is another way of soil nitrification for plant nutrition. It converts dinitrogen (N_2) into a form suitable for plant uptake, e.g., NH_4^+. Rhizospheric microorganisms provide the biological fixation of atmospheric nitrogen.

Phosphorus is second to nitrogen in terms of plant growth and development. It is an essential macronutrient for plant metabolism, i.e., cell division, energy production, macromolecule biosynthesis, membrane integrity, signal transduction, and photosynthesis [21]. Phosphorus is inherent to plant respiration. Unfortunately, most phosphorus compounds are insoluble and non-bioavailable [22].

The total phosphorus content in soil approximates 0.05% (w/w). Plants are able to absorb as little as 0.1% of bioavailable phosphorus because of such processes as cation precipitation in the soil, immobilization, adsorption, and interconversion to organic form [23]. As a result, phosphate fertilizers are an extremely popular means of continuous supply of phosphorus to plants. However, they possess a significant disadvantage: they tend to prepitate in the soil in great quantities, which is associciated with such adverse effects as accumulation of heavy metals, soil depletion, etc. Thus, crop farming needs a green approach that could provide the same effect as chemical fertilizers without negative consequences for the environment.

Microorganisms consume phosphorus in several ways, depending on the inaccessible forms of its compounds in the soil. They can solubilize inorganic phosphates by acidification, protonation, or chelation. They can also mineralize organic phosphates biochemically, e.g., via such enzymes as phosphatase, phytase, phosphonatase, and C-P-lyase [22].

Potassium, the third plant nutrient element, is abundant in agricultural soil. Potassium is important for photosynthesis: it produces adenosine triphosphate, transports sugar, water, and nutrients, and synthesizes starch, as well as participates in legume- and enzyme-based nitrogen fixation and protein synthesis. However, barely 5% of all potassium in soil is available for plant intake since 95% of potassium is bound with various minerals [24]. Potassium is present in soil in mineral (unavailable), soluble (available), non-exchangeable (fixed), and exchangeable forms. Fixed potassium remains a reserve source, whereas exchangeable potassium is easily absorbed by plant roots.

While potassium is not the most important element for plants, young plants need it even more than nitrogen and phosphorus [25]. Potassium improves their growth and development, as well as increases their resistance to diseases and stresses, e.g., drought, frost, pests, etc. In addition, it improves the quality of the crop and extends the shelf life of agricultural products. Potassium was found able to promote photosynthesis, which means it affects the formation of carbohydrates, fats, and proteins, regulates water absorption by plant roots, and helps shape a healthy root system [26]. Irrational and excessive use of potassium fertilizers reduces the yield, disrupts the microbial soil community, and causes groundwater pollution [27, 28]. Potassium solubilizing bacteria are a safe alternative to chemical fertilizers. Microbial potassium mobilizes and solubilizes insoluble potassium-containing minerals, e.g., mica, muscovite, feldspar, biotite, illite, orthoclase, etc. [29]. It also releases potassium compounds by producing oxalic, citric, tartaric, succinic, or acetic organic acids [30]. Figure 1 shows which organic acids appear as a result of the activity of potassium-solubilizing microorganisms.

The abovementioned acids of microbial production release potassium ion from potassium-containing minerals by chelating ions of Al^{3+}, Si^{4+}, Ca^{2+}, and Fe^{2+}. Some microorganisms form biofilms on the surface of minerals or stones. This film creates a controlled optimal microenvironment around the cells, which facilitates solubilization by organic acids and secondary metabolites. This mechanism sometimes lowers the pH of the rhizosphere: as a result, the potassium-containing mineral dissolves better and becomes more available for plant uptake.

Zinc facilitates carbohydrate and auxin metabolisms. In addition, it possesses antioxidant properties [31]. Zinc deficiency slows down shoot growth, as well as causes chlorosis, leaf-size reduction, withering, and fungal infections. Zinc also affects grain yield, pollen production, root development, absorption and transport of water, etc. Plants absorb zinc in the form of a divalent cation, which is present in soil in very small quantities. Other forms of zinc include insoluble complexes and minerals. Zinc

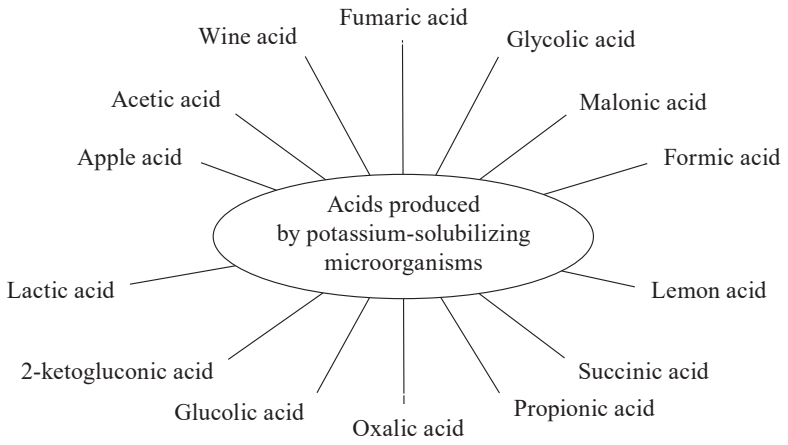

Figure 1 Organic acids involved in the release of potassium from potassium-containing minerals

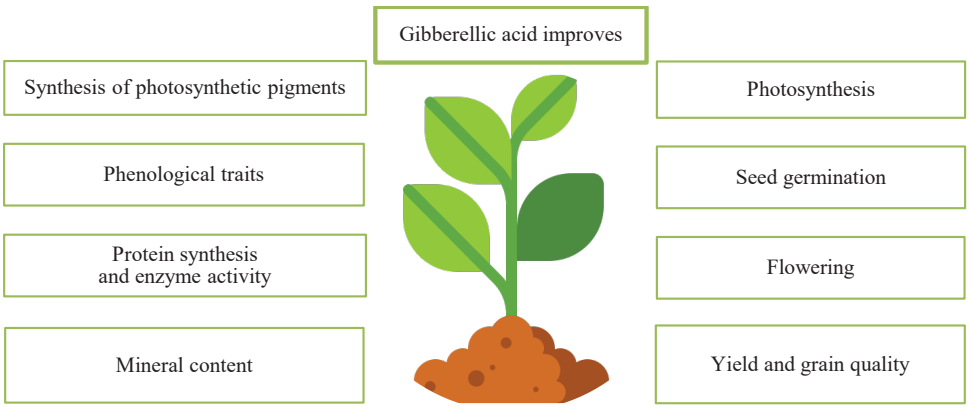

Figure 2 Effect of gibberellic acid on crops

is part of many fertilizers because zinc-poor agricultural products contribute to the development of zinc deficiency in people.

Zinc fertilizers have a long history. They include zinc sulfate, regular crop rotation, intercropping, cross-breeding, transgenic methods, and genetic engineering. However, all these methods are expensive, labor-intensive, and time-consuming, which makes zinc solubilizing microorganisms a prospective alternative. Zinc solubilizing microorganisms use a variety of strategies to convert zinc into a soluble form. For instance, they produce organic acids which bind zinc cations and reduce the pH in the immediate soil environment [32]. During acidifying, anions can also chelate zinc and increase its solubility. Other means of zinc solubilization include siderophores.

Siderophores are secondary low-molecular-weight metabolites with iron chelating properties. Iron is a vital element that participates in many biological processes, e.g., electron transport, oxygen metabolism, nitrogen xation, DNA and RNA synthesis, etc. [33–36]. Agricultural crops always experience serious iron deficiency because the content of available iron is negligible (10^{-9}–10^{-4} mol/L) and insufficient for plant growth and development. Most soils, especially alkaline ones, are extremely low in soluble iron, which is picomolar (10^{-9}–10^{-18} mol/L) [37]. Severe iron deficiency can cause plant death as early as at the seedling stage, thus reducing the yield. Siderophores use the transport mechanism of their cell membrane to transport iron ions. Metal ions combine with the siderophores produced by microorganisms in the soil around plant roots. While some complex compounds enter the cell membrane, metal ions remain in the periplasm and siderophores are released for cyclic use. Other complex compounds enter the cytoplasm through the cell membrane via the TonB mechanism [38]. Together with producing iron from insoluble hydroxide forms, siderophores also facilitate its release from iron citrate, iron phosphate, iron transferrin, iron in flavone pigment, sugars, and glycosides [39].

Growth regulators are important for plant development and protection. Gibberellic acid is an example of effective plant growth promoter. It is a phytohormone that affects the growth of roots and stems, as well as seed germination [40]. Figure 2 illustrates the role of gibberellic acid in plant growth and development.

Seed germination is an important stage controlled by environmental variables, e.g., light, humidity, temperature, etc., as well as by endogenous phytohormones, e.g.,

abscisic acid, gibberellic acid, etc. Gibberellic acid proved to inhibit the action of abscisic acid, a hormone that reduces the growth and development of seeds [41]. Endosperm cells cannot rapture without gibberellic acid, the level of which increases during swelling, which means that gibberellic acid is vital for root development.

Hydrogen cyanide is also important due to its toxic effect on plant pathogens. Hydrogen cyanide chelates metal ions and improves phosphate availability [41, 42]. As a product of bacterial synthesis, hydrogen cyanide is applied in the production of indolylacetic acid, antibiotics, and fluorescent insecticidal toxins, as well as in utilization of 1-aminocyclopropane-1-carboxylate deaminase [43].

The Kemerovo Region aka Kuzbass is an industrial region with numerous enterprises related to fuel and energy production, metallurgy, chemistry, coal mining, etc. Their anthropogenic effect threatens the quality of life of the population, not to mention the local biodiversity and biological soil capability [44, 45]. Industrial pollution leads to severe heavy-metal soil contamination. Heavy metals reduce the growth and productivity of agricultural plants and lower or even eliminate the effect of biological preparations. As a result, the local agricultural sector strives to develop safe methods of bioremediation of contaminated soil. At present, the local agricultural sector focuses on the concept of soil-protective and resource-saving agriculture to boost production volumes and improve the quality of agricultural products [46]. Some biofertilizers are based on extremophilic microorganisms isolated from polluted and disturbed soils [47]. These microorganisms have unique properties. In particular, some are able to accelerate plant growth and development. In addition, they are resistant to adverse environmental factors, e.g., heavy metals.

This research focused on extremophilic microorganisms isolated from coal dump soil to be used in biofertilizers that increase wheat yields.

STUDY OBJECTS AND METHODS

The study involved such extremophilic bacteria as *Klebsiella oxytoca*, *Rhizobium radiobacter*, and *Pseudomonas fluorescens*. They had been isolated from coal dump soil (53°26′ N; 87°25′ E) (Fig. 3) [48]. Microorganisms were isolated on a medium that contained salts of heavy metals, namely $CuSO_4$, $ZnSO_4$, $FeSO_4$, $CdCl_2$, $MgSO_4$, and $MnSO_4$.

The biocompatibility tests performed at the previous stage revealed several variants of bioconsortia (Table 1), which, together with individual extremophilic strains, became the objects of the current study.

Nitrogen fixation. The degree of nitrogen fixation was determined by spectrophotometry in Nfb nutrient medium at pH 6.5 [49]. The nutrient medium was sterilized at 121°C for 15 min.

To construct a calibration curve, we used 23 tubes with 2 mL sterile Nfb medium in each. Next, we added 0.93 N NH_4OH solution at the following quantities: 0.5, 1.0, 1.5, 2, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 11.0, and 12.0 µL. We used

Figure 3 Sampling coal dump

Table 1 Bioconsortia

Bioconsortium	*Achromobacter denitrificans*:*Klebsiella oxytoca*:*Rhizobium radiobacter* ratio
A	1:1:1
B	2:1:1
C	1:2:1
D	1:1:2

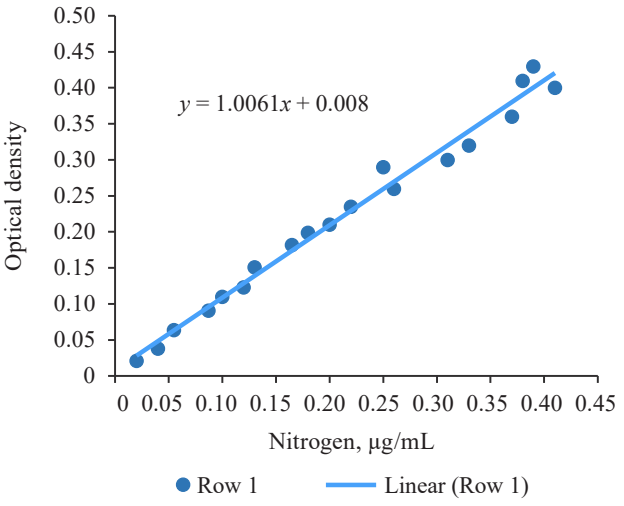

Figure 4 Standard absorbance curve

1.42 N HCl as a secondary standard. The total volume of each tube was adjusted to 3 mL with Nfb medium. We calculated the nitrogen concentration in each test tube based on the total volume and the volume of the NH_4OH solution we added. The optical density tests occurred at 610 nm using a UV 1800 spectrophotometer (Shimadzu, Japan). The measurements were carried out in triplicates to obtain a standard curve of how absorption depended on nitrogen concentration (Fig. 4).

We added 100 µL of inoculated bacterial strains and consortia to 4.9 mL of sterile Nfb medium and incubated at 25 ± 2°C for 48 h with constant stirring on an

LSI-3016A/LSI-3016R incubator shaker (Daihan Labtech, South Korea). Then, the tubes underwent centrifuging at 5000 rpm for 15 min. The optical density was measured at 610 nm. The sterile Nfb nutrient medium served as a control. The amount of nitrogen fixed by extremophilic microorganisms was obtained graphically using a curve that showed the dependence of nitrogen concentration in the nutrient medium on the optical density of standard solutions.

Measuring zinc solubilizing properties. This experiment involved spot inoculation of a daily bacterial culture/consortia onto Petri dishes with the following media: 1.00% of glucose, 0.10% $(NH_4)_2SO_4$, 0.02% KCl, 0.01% K_2HPO_4, 0.02% $MgSO_4$, 1.50% agar, 0.10% ZnO. The Petri dishes were incubated at 28 ± 2°C for 7 days. Clear inhibition zones around colony dots indicated that the extremophilic bacteria had some solubilizing ability. The efficiency of zinc solubilization was calculated as follows:

$$E_Z = \frac{D_{c+a}}{D_c} \times 100 \quad (1)$$

where E_Z is the efficiency of zinc solubilization, %; D_{c+a} is the diameter of the colony together with the inhibition zone, cm; D_c is the diameter of the colony, cm.

Determining potassium solubilizing properties. This experiment involved spot inoculation of a daily bacterial culture/consortia on Petri dishes with the following media: 1.000% of glucose, 0.500% $MgSO_4$, 0.005% $FeCl_3$, 0.100% $CaCO_3$, 2.000% $Ca_3(PO_4)_2$, 1.500% agar, and 5000% zeolite [30]. The Petri dishes were incubated at 28 ± 2°C overnight. Inhibition zones around colony dots indicated the ability of the culture to solubilize potassium. The efficiency of potassium solubilization was calculated as in Eq. (2):

$$E_K = \frac{D_{c+a}}{D_c} \times 100 \quad (2)$$

where E_K is the efficiency of potassium solubilization, %; D_{c+a} denotes the diameter of the colony together with the inhibition zone, cm; D_c stands for the colony diameter, cm.

Determining phosphate solubilizing properties. This experiment involved spot inoculation of a daily bacterial culture/consortia on Petri dishes with the following media: 5.00 g of $Ca_3(PO_4)_2$, 20.00 g glucose, 0.20 g NaCl, 0.10 g $MgSO_4$, 0.01 g $MnSO_4$, 0.01 g $Fe_2(SO_4)_3$, 15.00 g agar, and 1.0 L distilled water [50]. The Petri dishes were incubated for 4 days at 28 ± 2°C to form inhibition zones around colony dots. The efficiency of phosphate solubilization was determined as follows:

$$E_{ph} = \frac{D_{c+a}}{D_c} \times 100 \quad (3)$$

where E_{ph} is the efficiency of phosphate solubilization, %; D_{c+a} denotes the colony together with the inhibition zone, cm; D_c stands for the colony diameter, cm.

Producing gibberellic acid. We added 280 µL of 1 M $(CH_3COO)_2Zn$ and 10.6% $K_3[Fe(CN)_6]$ solution to 2 mL of culture liquid. After stirring, the culture liquid was centrifuged at 4500 rpm for 10 min. The resulting supernatant was mixed with 30% HCl at a ratio of 1:1. The solution was left to settle for 75 min. A similarly prepared nutrient medium served as control. The optical density was measured relative to 5% HCl using a spectrophotometer at a 254 nm wavelength [51]. The optical density of the sample was determined using the follow Eq. (4):

$$OD = OD_s - OD_c \quad (4)$$

where OD is the optical density; OD_s is the indicated optical density; OD_c denotes the optical density of the control sample.

The amount of synthesized gibberellic acid was determined using a calibration graph of a standard gibberellic acid solution between 10 and 200 µg/mL.

Obtaining siderophores. We added 100 µL of culture fluid to 100 µL of fresh Chrome Azurol S (CAS) reagent. The resulting solution was left to settle for 20 min. After that, the optical density was measured at 630 nm. A similarly prepared nutrient medium served as control [52]. The amount of siderophores synthesized was calculated as follows:

$$N_s = \frac{OD_c - OD_s}{OD_c} \quad (5)$$

where N_s is the amount of siderophores, %; OD_s stands for the optical density of the experimental sample; OD_c denotes the optical density of the control sample.

Producing hydrogen cyanide. This test involved a modified 4% nutrient agar medium with 4.4 g/L of amino acid L-glycine. We soaked filter paper in 0.5% picric acid in 1% Na_2CO_3 solution and applied it to the inner surface of the Petri dish lid. To synthesize hydrogen cyanide, we transferred bacterial colonies to plates with the modified 4% nutrient agar medium and uninoculated control. The Petri dishes were sealed with paraffin and incubated at 28 ± 1 °C until browning, which indicated hydrogen cyanide synthesis [53].

Effect of bacterial isolates and consortia on wheat growth. A suspension of the isolate in 2 mL of sterile distilled water was brought up to McFarland standard of 0.8–1.0 using a Densichek plus densitometer at 1.5×10^{-8} CFU/mL. Next, we added 1 mL of suspension to 10 mL of Luria Bertani nutrient medium and cultivated it on an incubator shaker at 28 ± 2°C and 110 rpm for 72 h.

Before seed inoculation, wheat seeds were sterilized with 2.5% NaClO for 3 min and washed three times with distilled water. After being planted in soil and watered with a suspension of bacterial isolates and consortia, the seeds were germinated for 10 days at 25°C and 50–60% humidity. Sterile control seeds were germinated for 10 days and watered with sterile distilled water.

Equation (6) made it possible to assess the germination rate of wheat seeds:

$$G = \frac{N_{gs}}{N_{ts}} \times 100 \quad (6)$$

where G means the germination rate, %; N_{gs} is the number of germinated seeds; N_{ts} stands for the total of seeds planted

The lengths of the roots and aerial parts of wheat were measured on graph paper with an accuracy of 0.5 mm.

All studies were triplicated. The obtained data values were expressed as the mean of three measurements with standard deviation. The statistical analysis involved Microsoft Office Excel 2007 and a one-sample paired Student's t-test for each pair. Differences were statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Nitrogen fixation. Table 2 shows the nitrogen-fixing capacity of extremophilic bacteria and bioconsortia A, B, C, and D.

Nitrogen fixation by extremophilic microorganisms ranged from 16.45 to 40.42 μg/mL nutrient medium. The data confirmed the results obtained for nitrogen-fixing properties of diazotrophic bacteria *Acinetobacter pitti*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Kosakonia oryzae* isolated from the rhizosphere of *Agave angustifolia* [49]. Their nitrogen-fixing capability was 18.34–42.06 μg/mL nutrient medium.

In this research, the best nitrogen fixation belonged to *Rhizobium radiobacter* at a nitrogen concentration of 30.86 μg/mL Nfb. As for the three-strain consortia, the best nitrogen fixation belonged to Consortium D with two shares of *R. radiobacter* at 40.42 μg/mL Nfb. It owed its nitrogen-fixing properties to the nitrogenase enzyme typical of *Rhizobium* sp. cultivated on NH_4^+ [54]. Other microorganisms in Consortium D also possessed certain nitrogen-fixing properties, which apparently enhanced the efficiency of nitrogen fixation. The Nfb medium changed color from green to blue as an indicator of bacteria with nitrogen-fixing ability. The color change was caused by bromothymol blue with its pH-dependent structure and color.

After the biological nitrogen fixation, ammonium ions accumulated in the nutrient medium and affected the pH. Two chemical forms can explain the effect of pH on the color of bromothymol blue. The quinoid form with one negative charge predominates in an alkaline environment, and it is responsible for yellow. A quinoid-phenolate structure with two negative charges predominates in an acidic environment and is associated with the blue color.

On average, consortia of extremophilic microorganisms showed better nitrogen fixation compared to individual bacterial strains. We recorded the worst result for *Klebsiella oxytoca*, which demonstrated a nitrogen-fixing capacity of 16.45 mg/mL Nfb. Apparently, the low result is connected with the poor ability of this microorganism to produce nitrogenase.

Determining zinc solubilizing properties. Table 3 illustrates the zinc-solubilizing potential of extremophilic isolates and bioconsortia.

The efficiency of zinc solubilization by soil bacteria and their consortia was 135–182%. *Bacillus megaterium* AN24, *Bacillus aryabhattai* AN30, *B. megaterium* AN31 and AN35 are routinely used in agriculture as growth promoters. Their zinc solubilization efficiency was reported as 120–258% [55]. Extremophilic microorganisms demonstrated zinc solubilization properties similar to those of growth-stimulating microorganisms.

Consortium D with a double share of *R. radiobacter* demonstrated the best zinc solubilization potential of 182.34% while Consortium B with two shares of *Achromobacter denitrificans* had the lowest result for the consortia samples (163.61%). As for individual extremophilic isolates, *R. radiobacter* proved to be the best zinc-solubilizer (154.36%). However, this achievement was far below the lowest result in the consortia group. Probably, *R. radiobacter* produced a lot of anionic organic acids, e.g., gluconic and *α*-ketogluconic, which could chelate zinc through carboxyl and hydroxyl groups, thus increasing its solubility and improving the mineral uptake by the plant [56].

Determining potassium solubilization properties. Table 4 demonstrates the potassium solubilizing capability for the soil extremophilic microorganisms and their consortia.

Table 2 Nitrogen-fixing ability of extremophilic bacteria and their consortia based

Sample	Nitrogen, μg/mL Nfb
Achromobacter denitrificans	20.32 ± 0.02
Klebsiella oxytoca	16.45 ± 0.06
Rhizobium radiobacter	30.86 ± 0.10
Consortium A (1:1:1)	24.84 ± 0.01
Consortium B (2:1:1)	38.41 ± 0.03
Consortium C (1:2:1)	35.81 ± 0.05
Consortium D (1:1:2)	**40.42 ± 0.06**

Table 3 Zinc solubilization properties of extremophilic bacteria and their consortia

Sample	Colony diameter, mm	Colony diameter + inhibition zone, mm	Zink solubilization, %
Achromobacter enitrificans	6.13 ± 0.05	8.32 ± 0.09	135.73 ± 0.14
Klebsiella oxytoca	7.36 ± 0.12	10.87 ± 0.04	147.69 ± 0.04
Rhizobium radiobacter	7.23 ± 0.11	11.16 ± 0.06	154.36 ± 0.07
Consortium A (1:1:1)	5.32 ± 0.03	9.52 ± 0.02	178.95 ± 0.05
Consortium B (2:1:1)	6.32 ± 0.04	10.34 ± 0.11	163.61 ± 0.14
Consortium C (1:2:1)	6.89 ± 0.11	11.83 ± 0.15	171.70 ± 0.12
Consortium D (1:1:2)	**6.23 ± 0.05**	**11.36 ± 0.02**	**182.34 ± 0.11**

Table 4 Potassium solubilization properties of extremophilic bacteria and their consortia

Sample	Colony diameter, mm	Colony diameter + inhibition zone, mm	Potassium solubilization, %
Achromobacter denitrificans	5.36 ± 0.10	8.63 ± 0.11	161.01 ± 0.01
Klebsiella oxytoca	6.43 ± 0.03	8.26 ± 0.02	128.46 ± 0.03
Rhizobium radiobacter	**6.52 ± 0.04**	**12.58 ± 0.03**	**192.94 ± 0.05**
Consortium A (1:1:1)	6.12 ± 0.11	10.78 ± 0.04	176.14 ± 0.05
Consortium B (2:1:1)	7.10 ± 0.13	11.59 ± 0.04	163.24 ± 0.07
Consortium C (1:2:1)	7.30 ± 0.06	13.65 ± 0.09	186.99 ± 0.03
Consortium D (1:1:2)	7.10 ± 0.08	13.10 ± 0.07	184.51 ± 0.04

Table 5 Phosphate solubilization properties of extremophilic bacteria and their consortia

Sample	Colony diameter, mm	Colony diameter + inhibition zone, mm	Phosphate solubilization, %
Achromobacter denitrificans	**3.21 ± 0.04**	**6.32 ± 0.05**	**196.88 ± 0.01**
Klebsiella oxytoca	2.62 ± 0.02	5.12 ± 0.02	195.42 ± 0.02
Rhizobium radiobacter	2.68 ± 0.14	3.65 ± 0.06	136.19 ± 0.09
Consortium A (1:1:1)	3.67 ± 0.01	4.56 ± 0.07	124.25 ± 0.05
Consortium B (2:1:1)	2.37 ± 0.03	2.87 ± 0.01	121.10 ± 0.04
Consortium C (1:2:1)	1.61 ± 0.08	1.80 ± 0.09	111.80 ± 0.03
Consortium D (1:1:2)	3.11 ± 0.01	4.32 ± 0.06	138.91 ± 0.05

Efficiency of potassium solubilization ranged from 128 to 193%. These data were slightly lower than those published by Jabin *et al.*, who obtained 185.00–257.32% for isolates of *Bacillus*, *Pseudomonas*, and *Sinorhizobium* [57].

R. radiobacter demonstrated the best potential for potassium solubilization (192.94%). Presumably, this strain produced organic acids, e.g., gluconic, oxalic, α-ketogluconic, succinic, or citric, which dissolved the mineral potassium by protonation and acidification [56]. *K. oxytoca* had the lowest results of 128.46%, which also proved to be the lowest among all the study objects due to its failure to produce organic acids.

Determining phosphate solubilization properties. Table 5 illustrates the potential of soil bacteria and their consortia for phosphate solubilization.

Efficiency of phosphate solubilization ranged from 111.80 to 196.88%. Blanco-Vargas *et al.* studied a consortium of *Pseudomonas* sp. and *Serratia* sp., isolated from Colombia's soil and achieved phosphate solubilization indices of 210 and 200%, respectively [59].

In this research, *A. denitrificans* proved to be the best phosphate-solubilizer with 196.88%. It produced a lot of organic acid, e.g., oxalic, gluconic, acetic, malic, etc., which acidified the environment and dissolved phosphorus [60]. Unlike individual isolates, all consortia showed poor phosphate solubilization. Obviously, extremophilic symbioses are inefficient as phosphate solubilizers. Consortium D with a double share of *R. radiobacter* had the highest solubilization efficiency (138.91%), which was as high as the lowest solubilization efficiency for individual isolates, i.e., *R. radiobacter* with its 136.19%. Consortium C had the lowest phosphate solubilization of 111.80%.

Producing gibberellic acid. Table 6 illustrates the potential of soil bacteria and their consortia for gibberellic acid production.

All the samples demonstrated gibberellic acid production potential between 475.00 and 611.50 μg/mL.

Table 6 Gibberellic acid production potential of extremophilic bacteria and their consortia

Sample	Gibberellic acid, μg/mL
Achromobacter denitrificans	475.00 ± 0.50
Klebsiella oxytoca	**611.50 ± 0.31**
Rhizobium radiobacter	601.50 ± 0.06
Consortium A (1:1:1)	551.50 ± 0.12
Consortium B (2:1:1)	479.00 ± 0.13
Consortium C (1:2:1)	589.00 ± 0.24
Consortium D (1:1:2)	581.50 ± 0.38

Table 7 Siderophore production by extremophilic soil microorganisms and their consortia

Sample	Siderophores, %
Achromobacter denitrificans	60.25 ± 0.03
Klebsiella oxytoca	28.69 ± 0.05
Rhizobium radiobacter	53.36 ± 0.08
Consortium A (1:1:1)	67.82 ± 0.07
Consortium B (2:1:1)	57.43 ± 0.03
Consortium C (1:2:1)	**82.61 ± 0.03**
Consortium D (1:1:2)	71.32 ± 0.03

Kaur *et al.*, who isolated and tested bacteria from natural sources in India, reported 550 μg/mL [61]. In another study, *Microbacterium laevaniformans* RS0111 produced 67.23 μg/mL [62].

The largest amount of gibberellic acid belonged to *K. oxytoca* and reached 611.50 μg/mL. *A. denitrificans* had the lowest result of 475 μg/mL. As for the consortia group, Consortium C with a double share of *K. oxytoca* appeared to be the most efficient gibberellic acid producer, yielding 589 μg/mL.

Producing siderophores. Table 7 sums up the percentage of siderophores produced by soil bacteria and their consortia.

Table 8 Hydrogen cyanide production by extremophilic soil microorganisms and their consortia

Sample	Hydrogen cyanide
Achromobacter denitrificans	–
Klebsiella oxytoca	+
Rhizobium radiobacter	+
Consortium A (1:1:1)	++
Consortium B (2:1:1)	+
Consortium C (1:2:1)	++
Consortium D (1:1:2)	+

"+" Moderate hydrogen cyanide production; "++" active hydrogen cyanide production; "–" no hydrogen cyanide production

The samples demonstrated siderophore production properties in the range from 28.69 to 82.61%. The rhizobacterial strain of *Pantoea dispersa* was reported to produce 70.54% siderophores [50]. Li *et al.* studied inoculants of *Paenibacillus tundrae*, *Bacillus mycoides*, and *Brevibacterium frigoritolerans* isolated from the soil of the Qinghai-Tibet Plateau, and they proved to be efficient siderophore producers (89.58–94.74%) [63].

In this research, Consortium C with two shares of *K. oxytoca* showed the best potential for siderophore production (82.61%). The medium turned from blue to yellow-pink as siderophores chelated. Individual isolates of extremophilic microorganisms performed poorly, compared to bacterial consortia. *K. oxytoca* demonstrated the lowest potential for siderophore production (28.69%). Solutions with Hg^{2+} and Ag^{2+} demonstrated a subtle color change from blue to sunset yellow, which resulted in weak siderophore binding to Hg^{2+} and Ag^{2+} [64]. As for the isolates, *A. denitrificans* demonstrated the best indicator of 60.25%.

Producing hydrogen cyanide. Table 8 displays the ability of bacterial isolates and consortia to produce hydrogen cyanide.

All the samples but *A. denitrificans* were able to produce hydrogen cyanide. Consortia A (with all isolates in equal shares) and C (with two shares of *K. oxytoca*) were quite efficient in this respect while *R. radiobacter*, *K. oxytoca*, Consortium B (with a double share of *A. denitrificans*), and Consortium D (with a double share of *R. radiobacter*) demonstrated moderate results. Therefore, isolates *K. oxytoca* and *R. radiobacter*, as well as all the consortia, could protect agricultural plants from diseases caused by phytopathogenic fungi [53]. These findings corresponded with those published by Mowafy *et al.*, who reported the ability of microorganisms of the *Rhizobium* genus to produce hydrogen cyanide [65]. Similarly, Walpola *et al.* found *K. oxytoca* capable of producing hydrogen cyanide [66].

Effect of bacterial isolates and consortia on wheat growth. Figure 5 illustrates the wheat germination results.

On experiment day 10, all extremophilic bacteria and consortia increased the germination rate of wheat seeds compared to the control samples. The germination of control wheat samples, which received distilled water, stayed below 75%. The best seed germination rates belonged to Consortium C (with a double share of *K. oxytoca*), which showed the best results for siderophores and hydrogen cyanide. Consortium-treated samples demonstrated 84% germination. *K. oxytoca* had the lowest effect on germination rate (76%).

Figure 6 shows the average length of wheat roots.

All the bacterial isolates and consortia were able to increase the length of wheat roots. The average root length of control wheat samples was 80.3 mm. Consortia C (with a double share of *K. oxytoca*) and B (with a

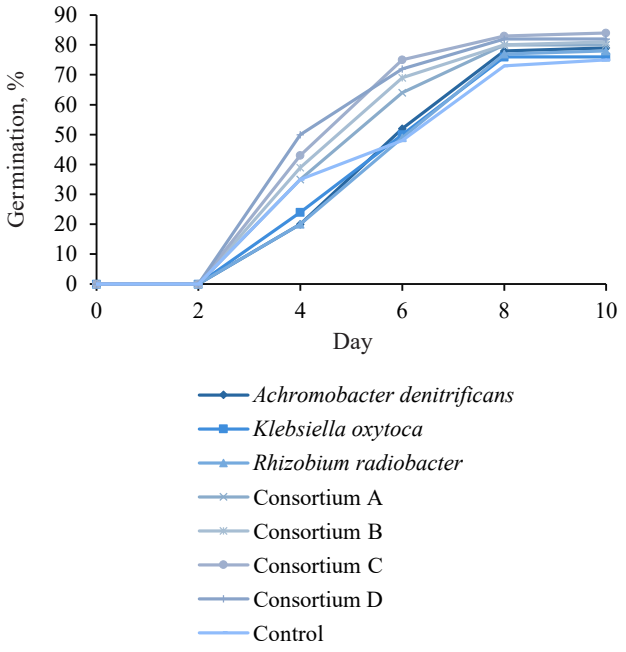

Figure 5 Wheat germination

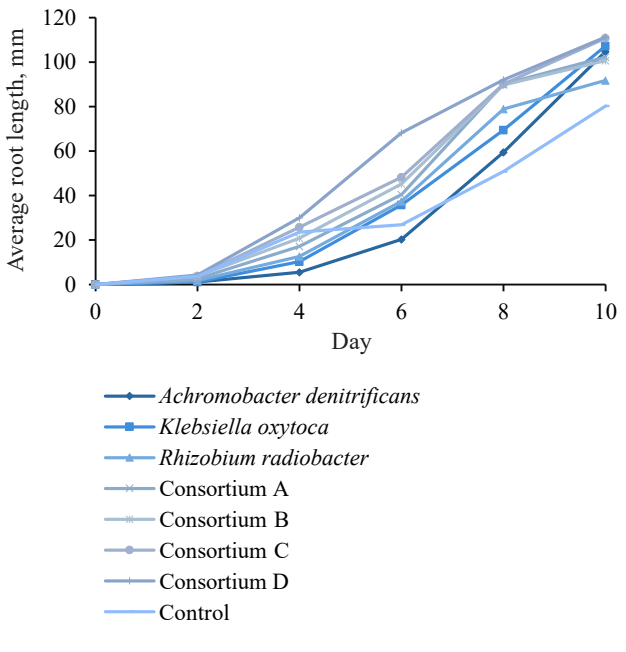

Figure 6 Effect of extremophilic bacteria and consortia on average root length

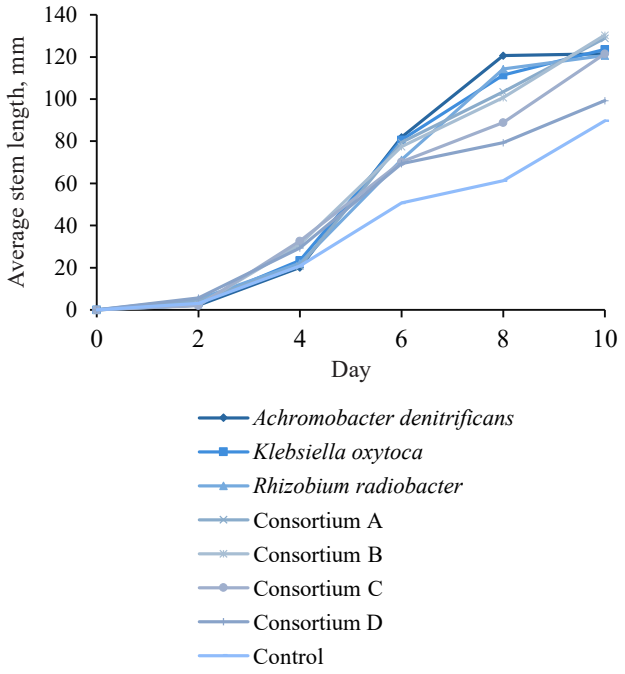

Figure 7 Effect of extremophilic bacteria and consortia on average stem length

double share of *A. denitrificans*) had the greatest positive effect on root length: 110.8 and 111.3 mm, respectively. *R. radiobacter* proved least effective in stimulating wheat roots (91.8 mm).

Figure 7 shows the average length of wheat stems.

The extremophilic soil bacterial isolates and consortia were able to stimulate stem growth in wheat. The average stem length in the control wheat samples was 89.7 mm. Consortium B (with a double share of *A. denitrificans*) produced the longest average stem (130.4 mm). A similar result of 128.9 mm belonged to Consortium A, where all the isolates were represented in equal shares. As for the bacterial isolates, *K. oxytoca* proved to be the most effective strain with 123.6 mm of average stem length. Consortium D (two shares of *R. radiobacter*) with its 99.3 mm proved to be the least effective sample.

In this research, experimental microorganisms and their consortia demonstrated their efficiency in nitrogen fixation, solubilization of zinc, potassium, and phosphates, as well as proved to be descent hydrogen cyanide and gibberellic acid producers. These properties had a positive effect on the growth and development of wheat seeds, as evidenced by germination rate and stem and root lengths.

Rhizobacteria with plant-growth promoting properties increase the growth and development of agricultural crops. They improve the overall health of plants by promoting nutrient uptake, protecting against phytopathogenic microbes, and increasing resistance to various abiotic stresses [67]. Rhizobacteria are capable of producing phytohormones, e.g., gibberellic acid, as well as siderophores. They solubilize phosphates, zinc, and potassium. In addition, they fix nitrogen, which improves plant growth and fertility. Some extremophilic microorganisms demonstrate rhizobacterial potential due to their ability to produce secondary metabolites and enzymes that are of great commercial interest to many industries, e.g., agriculture [68]. Finally, extremophiles are able to survive under aggressive environmental conditions. As a result, products that contain extremophiles have a better storage capacity. Extremophilic microorganisms maintain their effectiveness in polluted areas.

In this study, soil microorganisms isolated in the Kemerovo Region demonstrated plant growth-promoting properties and potential for agricultural use.

Due to these properties, extremophilic microorganisms isolated from disturbed areas and their consortia were able to increase seed germination from 76 to 84%. The stem length in the experimental wheat increased by 24–45%, while the average root length grew by 14–39%.

Research prospects include the enzyme complex of extremophilic isolates and their consortia. We plan a qualitative and quantitative analysis of metabolites that render microorganisms their growth-stimulating properties. A set of chromatographic methods will make it possible to develop biofertilizers that will improve the quality of agricultural crops and ensure food security.

CONCLUSION

Bacterial isolates of *Achromobacter denitrificans*, *Klebsiella oxytoca*, and *Rhizobium radiobacter*, as well as their consortia were able to improve the growth and development of wheat seeds. They proved to be efficient nitrogen fixators, solubilizers of phosphates, zinc, and potassium, and producers of siderophores, hydrogen cyanide, and gibberellic acid. The best nitrogen fixation properties belonged to *R. radiobacter* and reached a nitrogen concentration of 30.86 μg/mL Nfb. The best ability to solubilize zinc solubilization efficiency of 182.34% was observed in Consortium D with a double share of the same isolate. *R. radiobacter* also was the most efficient sample in potassium solubilization (192.94%) while *A. denitrificans* was the most efficient phosphate solubilizer (196.88%). *K. oxytoca* produced the largest amount of gibberellic acid (611.50 μg/mL). Consortium C with a double share of *K. oxytoca* was the most efficient siderophore producer (82.61%). All the samples were good at hydrogen cyanide production, with the exception of *A. denitrificans*. The best seed germination rate of 84% belonged to Consortium C. Consortium C (with a double share of *K. oxytoca*) and Consortium B (with a double share of *A. denitrificans*) had the greatest positive effect on root length: 110.8 and 111.3 mm, respectively. Consortium B was also responsible for the longest average stem length (130.4 mm).

Extremophilic microorganisms isolated from disturbed soils of the coal-mining Kemerovo Region and their consortia improved the growth and development of wheat. They proved to be a promising source of biofertilizers that improve food security and quality of agricultural crops.

CONTRIBUTION

The authors contributed to the manuscript equally and are equally responsible for any potential plagiarism.

CONFLICT OF INTEREST

The authors declared no conflict of interests regarding the publication of this article.

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