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Study of soil rhizobacteria from Chernevaya Taiga in Western Siberia and their potential effect on plant growth

Estudio de las rhizobacterias del suelo de Chernevaya Taiga en Siberia Occidental y su efecto potencial en el crecimiento de las plantas

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ABSTRACT

The isolation of soil bacteria from various environments with exceptionally high fertility rates represents an opportunity to identify promising agents to promote agriculture production. The paper aims to study the densities of cultivable bacteria and isolate bacteria from the radish and spring wheat rhizosphere. Plants were grown in the pot experiment with virgin Chernevaya soil, which possessed extraordinary productivity, and zonal forest soil that did not demonstrate similar features. Fifty-nine bacterial isolates were purified and evaluated for their beneficial effects on the early growth of wheat. Isolates belonged to Proteobacteria, Actinobacteria, and Firmicutes phyla, and the most represented genera were *Pseudomonas*, *Streptomyces*, *Paenibacillus*, and *Methylobacterium*. These dominant bacteria were used in plant inoculation studies. Strains promoted a significant increase in shoot and root length and biomass, thus they may be considered plant growth-promoting rhizobacteria [PGPR]. Regarding biotest assays, strains that displayed high plant growth-promoting capabilities [PGP] were selected for further investigation. This study contributed to bacteria isolates from a unique natural environment with biotechnological potentials in improving plant growth and showed potency to be exploited as bioinoculants.

Keywords: Rhizosphere, Plant productivity, Plant growth, Promoting bacteria (PGPB), Chernevaya soil

RESUMEN

El aislamiento de bacterias del suelo de varios ambientes con tasas de fertilidad excepcionalmente altas representa una oportunidad para identificar agentes prometedores para promover la producción agrícola. El artículo tiene como objetivo estudiar las densidades de las densidades bacterianas cultivables y aislar bacterias de la rizosfera de rábano y trigo de primavera. Las plantas se cultivaron en el experimento de la maceta con suelo virgen Chernevaya, que poseía una extraordinaria productividad, y suelo de bosque zonal que no mostraba características similares. Cincuenta y nueve aislados bacterianos fueron purificados y evaluados por sus efectos beneficiosos sobre el crecimiento temprano del trigo. Los aislamientos pertenecieron a los filos Proteobacteria, Actinobacteria y Firmicutes, y los géneros más representados fueron Pseudomonas, Streptomyces, Paenibacillus y Methylobacterium. Estas bacterias dominantes se utilizaron en estudios de inoculación de plantas. Las cepas promovieron un aumento significativo en la longitud y la biomasa de brotes y raíces, por lo que pueden considerarse rizobacterias promotoras del crecimiento vegetal [PGPR]. Con respecto a los ensayos de bioensayo, se seleccionaron cepas que mostraron altas capacidades de promoción del crecimiento de plantas [PGP] para una mayor investigación. Este estudio contribuyó a aislar bacterias de un entorno natural único con potencial biotecnológico para mejorar el crecimiento de las plantas y mostró potencia para ser explotadas como bioinoculantes.

Palabras clave: rizosfera, productividad vegetal, bacterias promotoras del crecimiento vegetal (PGPB), suelo Chernevaya.

1. INTRODUCTION

The agriculture sector today is expected to move towards environmentally sustainable development while increasing its productivity and conserving natural resources and the environment. In recent years, there has been an increased interest in studying beneficial microorganisms in bulk soil and rhizosphere in the natural environment, which can be exploited to increase crop productivity.

Currently, only 3% of the world's land remains ecologically intact. Chernevaya taiga is an example of such fragments of wilderness, an undamaged environment in east Siberian forests. It can be described as "an ecological island" - an area isolated by natural means from the surrounding land and a natural micro-habitat. Chernevaya taiga is a boreal formation at elevations ranging from 400 to 900 m above sea level in hyper humid sectors of the Altai-Sayan region. The soil never freezes because of much snow in the wintertime (Abakumov et al., 2020). Chernevaya taiga is an area with exceptional biodiversity, which is called the "Siberian jungle." These forests are distinguished by relict species of flora, plants' gigantism, and the high-speed circulation of substances in the ecosystem. The high intensity of biological processes is attributed to the local hydrothermal and climatic conditions and the functional structure of the communities of living organisms.

As the essential and active component of various ecosystems, rhizosphere microorganisms promote plant nutrient absorption, defend against pests and pathogens, and enhance different types of tolerance to resistance to non-biological or biological stress (Gupta, Parihar, Ahirwar, Snehi & Singh, 2015; Kumar & Dubey, 2020). Plants modulate the soil microbiota by root exudation, assembling a complex rhizosphere microbiome with organisms spanning different trophic levels. Plants can modulate the microbiome assembly in the rhizosphere (Hartmann, Schmid, Van Tuinen & Berg, 2009). Besides functioning as substrates for microbial growth, root exudates contain signaling molecules, microbial attractants, stimulants, and inhibitors or repellents (Baetz & Martinoia, 2014).

Knowledge of the distribution and diversity of indigenous bacteria in the natural ecosystem with high fertility, isolation, and characterization can be the basis for creating agricultural technology based on the novel specific plant growth-promoting rhizobacteria [PGPR] strains. It can be used as a growth-promoting or enhancing inoculum to achieve desired crop production. Research has been carried out based on PGPR use in agriculture to improve the yield of different vegetables and cereals (Deepa, Dastager & Pandey, 2010; Flores-Vargas & O'Hara, 2010). However, the isolation and application of PGPR from the unique environment with high productivity represent a relatively new scenario. Recently, metagenomic studies of the soils of the Chernevaya taiga were carried out, and significant differences were found in the taxonomic composition of microorganisms compared with soils from surrounding forest areas (Abakumov et al., 2020).

2. MATERIALS AND METHODS

This study aimed to isolate PGPR bacteria from Chernevaya soil possessing extraordinary productivity. We hypothesized that by growing cultivated plants on the soil of Chernevaya taiga that has never been used in agriculture practice, it would be possible to isolate novel promising RGPR root-associated bacteria. The main objectives of the study were (1) to assess the number and diversity of bacteria in the rhizosphere of radish and spring wheat grown in the pot experiment, (2) to isolate bacteria unique to the plants grown on the Chernevaya soil, and (3) to evaluate the photostimulation activity of bacterial isolates.

Two environments were selected for this study. We conducted the field study during the summer season of 2019 on the territory of the Tomsk Region. According to the World Reference Base of Soil Resources rules, a typical undisturbed ecosystem of Chernevaya taiga (T1) is represented by Retisols of various parent loams and clays materials. The control oligotrophic zonal forest environment (T3) is represented by primary soils formed on sands of aeolian genesis. From both sites, bulk soil samples were taken in quadruplicate at 10 cm depth to avoid contamination with other surfaces. Each sample was placed in sterile plastic bags and immediately stored in the cold.

The pot experiment was conducted in the room under 16 hours of daylight and 8 hours of darkness photoperiod. The minimum and maximum temperatures during the experiment ranged from 22.0 °C to 23.0 °C. Seeds of radish (*Raphanus raphanistrum subsp. sativus*) varieties Red light and spring wheat (*Triticum*.) varieties Lada were sterilized according to (Lindsey, Rivero, Calhoun, Grotewold & Brkljacic, 2017) and germinated on filter paper moistened with sterile deionized water in the dark for approximately two days. We transferred the seedlings to pots filled with soil. The experimental design included the Chernevaya bulk soil and forest soils in three replicates. Each replicate was composed of a pot with ten plants. We measured and calculated the oven-dried (70 °C, 72 hours) plant biomass at harvest (plant growth parameters). We monitored the shoot ratio during the plant growth period (root and shoot height).

For rhizosphere sampling, we harvested the whole root system by carefully removing the plants from pots and gently shaking them to remove excess soil adhered to the root system. Then, soil closely associated with plant roots (rhizoplane) was separated by a vigorous vortex with sterile PBS solution. The endosphere sample was received after root surface sterilization and grinding.

We used two cultures in both sites to obtain bacterial isolates: soil agar and Luria–Bertani [LB] agar. Bacteria densities were determined at ten days in the serial dilution plating technique, and the number of viable cells was estimated as colony-forming units [CFU] number.

Bacterial colonies from T1 soil morphologically different from T3 colonies were isolated and characterized. All strains classified as distinctive were stored in glycerol at -80°C, creating a culture collection of bacteria from this unique environment.

3. RESULTS

The plant physiological parameters of plants grown on the Chernevaya soil were significantly higher compared to the control forest soil (Table 1).

As expected, a clear difference between root-associated bacterial densities in Chernevaya and forest soil was observed (Table 2). A total of 32 bacteria were isolated from radish and 27 from wheat. Hence, the most abundant phyla in both sites corresponded to Proteobacteria, Firmicutes, and Actinobacteria. Regarding genera isolated, *Pseudomonas* and *Paenibacillus* were the most represented genus in our study, with 22 isolates from radish (56.4%) and 11 isolates from the wheat rhizosphere. We also observed the presence of less meant but specific genera, such as *Azospirillum* and *Methylobacterium*.

Table 1: Measurement of plant physiological parameters for radish and wheat are grown in the pot experiments using Chernevaya and control soils

Culture	Parameters	Chernevaya soil	Control soil
Radish	Shoot length, mm	80±11	50±5
	Root length, mm	70±8	35±4
	Root: shoot ratio	0.88	0.70
	Plant dry mass, mg $plant^{-1}$	16.63±0.4	15.47±0.3
Wheat	Shoot length, mm	19.7±3.3	16.3±2
	Root length, mm	46±1.3	37±2.0
	Root: shoot ratio	0.41	0.43
	Plant dry mass, mg $plant^{-1}$	140±0.05	90±0.03

Table 2: Bacterial density associated with root zone of radish and wheat is grown in the pot experiments with Chernevaya and control soil

Culture	Parameters	Chernevaya soil	Control soil
Colony-forming units (CFU) number			
Radish	Rhizosphere	9.5×10^{8}	6.8×10^{8}
	Rhizoplane	7.5×10^{8}	2.0×10^{8}
	Endosphere	1.0×10^{9}	4.7×10^{9}
Wheat	Rhizosphere	8.1×10^{7}	1.0×10^{8}
	Rhizoplane	2.8×10^{8}	7.1×10^{7}
	Endosphere	3.0×10^{8}	2.0×10^{8}

We studied the unique isolates using the biotest method with wheat seedlings. Hence, four bacterial cultures (e.g., *Azospirillum*, *Methylobacterium*, *Paenibacillus*, *Streptomyces*) increased the growth parameters and biomass of wheat seedlings. The length of the stems of the seedlings increased by 1.2-1.5 times, the length of the roots by 1.6-2.2 times, and the weight of the seedlings by 1.4-1.7 times.

4. DISCUSSION

The rhizosphere is a root zone considered a hotspot for bacterial diversity, where bacteria are mainly expressed in functions adapted to the root's presence and promote plant growth. Continuous study of the natural biodiversity of soil microorganisms and the optimization and regulation of microbial interactions in the rhizosphere of crops represents a step toward developing more effective bioinoculants.

The active release of various organic compounds by the roots of plants provides nutrients to the soil microorganisms, creating favorable conditions for their existence in the rhizosphere zone. The phenomenon of a higher density of microorganisms in the root zone is called "the rhizosphere effect" and is characterized by the R/S (rhizosphere/soil) ratio. For bacteria, this value varies from 10 to 100 (Beneduzi, Ambrosini &

Passaglia, 2012; Baetz & Martinoia, 2014). According to current data, most rhizosphere bacteria belong to the Actinobacteria, Bacteroides, Firmicutes, and Proteobacteria (Chahboune, Barrijal, Moreno & Bedmar, 2011), and our results are in line with these findings.

Our study revealed significant differences in the number and diversity of rhizosphere bacteria in the plants grown on Chernevaya and oligotrophic zonal soil. Unfortunately, we are not aware of similar work comparing undisturbed soils. However, rhizosphere bacterial diversity was significantly lower under monoculture (low fertility) than mixture planting (Hartmann et al., 2009).

Root-related microbiota plays an essential role in stimulating plant growth and development. Such microbial communities affect the root and the entire plant. In our study, all bacterial isolates significantly increased shoot and root length and dry weight. Plant growth stimulation may result from the positive effects on plant growth through direct or indirect mechanisms. Their key is nitrogen fixation, phytohormone production, and nutrient mobilization (Hartmann et al., 2009). We cannot determine what mechanisms were present in each of the cases. However, we can make assumptions based on the literature data. For example, *Azospirillum* and *Paeniacillus* are active nitrogen-fixing bacteria and are widely used as bio-inoculants (Hakim et al., 2021). *Azospirillu*m and *Methylobacterium* are known to synthesize different phytohormones, including auxins, cytokinins, and gibberellins (Olenska et al., 2020). *Streptomyces* is a perspective biocontrol agent of phytopathogenic microbes (Karthika, Midhun & Jisha, 2020).

5. CONCLUSION

In an era of climate change, the identification, isolation, and characterization of bacteria with PGPR properties from unique environments represent excellent and novel biotechnological tools. Furthermore, soil for vegetable cultivation is limited. Therefore, using these bacteria would improve the tolerance, productivity, and yield of plants of agronomic interest which is a problem for world food security.

We have demonstrated that rhizospheric microbiota of agricultural plants (radish and spring wheat) grown on Chernevaya soil differs significantly from corresponding microbiota on the control soil that does not possess high fertility. Furthermore, four bacterial cultures, inherent for Chernevaya taiga (*Azospirillum*, *Methylobacterium*, *Paenibacillus*, *Streptomyces*), promote the reliable increase of growth parameters and biomass of the wheat seedlings. This promising result supports the idea that the bacteria, specific to Chernevaya taiga, influence high soil fertility; a more detailed analysis of individual strains' roles and their combinations will shed light on this effect.

Thus, bacterial isolates from the Chernevaya soil have a significant photostimulation effect and are of interest in creating plant-microbial associations for environmentally friendly agriculture. This fact characterizes them as a suitable object for studying microbial factors of fertility and the development of innovative technologies for increasing the productivity of soils and crops. Research indicates the potential of these bacterial cultures for creating plant-microbial associations used as bioinoculants for wheat and other crops in the field.

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