Isolation and characterization of endophytic bacterial community from potato (*Solanum tuberosum l.)* of five varieties

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Abstract. Plant microbiota plays a crucial role in the growth, development, and health of their hosts. Understanding the plant–microbe interactions support strategies to improve the productivity and quality of crops. This study aimed to analyze the cultured endophytic bacteria from seed potato of five varieties. The morphological diversity of isolates was assessed using the following parameters: color, form, surface, appearance, size of the colony, cell shape, Gram reaction, endospore formation. The identification of the endophytic bacteria was carried out using MALDI-TOF mass-spectrometry method. A total of 49 endophytic isolates were first found in leaves, stems, roots and tubers of potato cultivars Reggi, Sprint, Gala, Red Sonia, and Picasso at the flowering stage. It was shown that the majority of endophytic bacteria (59%) belonged to the genera *Bacillus*. Functional diversity was screened for the ability to produce the phytohormone indole-3-acetic acid (IAA) and antagonistic potential against *Fusarium oxysporum*. The application of cluster analysis methods to the morphological and functional characteristics of 49 endophytic isolates allowed for a clear demonstration of the diversity of endophytic bacteria in the different potato varieties studied. The most interesting cluster was found to be cluster 2, which contains the isolates *Bacillus toyonensis* R SE L 22 and *Enterobacter ludwigii* R S R 3 obtained from the leaves and roots of cultivar Reggi. These isolates showed the highest IAA production, inhibited the growth of strain *F. oxysporum* DR57 by 62.2% and 50.3%, respectively, and can be considered as promising growth-promoting endophytic bacteria.

1 Introduction

The cultivation of major crops around the world still relies on the use of huge amounts of pesticides, fertilizers etc. Most of the chemicals used contaminate the ecosystem and have a serious effect on human health. Therefore, sustainable agriculture should be developed based

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on new environmentally friendly technologies to increase yields in organic crop production. Over the past two decades, there has been a growing body of research focused on endophytic microorganisms, which asymptomatically colonize the internal tissues of plants [1]. These endophytes establish complex associations with their hosts and contribute to plant fitness through multiple physiological and biochemical mechanisms. Specifically, endophytic microorganisms facilitate the acquisition of essential macronutrients, modulate osmotic potential and stomatal conductance, influence root system architecture, and enhance tolerance to various abiotic stressors. In addition, they exhibit antagonistic activity against phytopathogens and play a pivotal role in the activation of plant defense responses [2].

The potato (*Solanum tuberosum* L.) crop represents one of the most economically and nutritionally significant crops worldwide, cultivated in more than 160 countries and serving as a staple food for approximately one billion people [3]. Russia is currently one of the largest potato producers globally. Despite the crop's potential productivity exceeding 50 metric tonnes per hectare, the national average yield remains comparatively low, at approximately 14-16 metric tonnes per hectare. This discrepancy is largely attributed to the use of substandard seed material and the high incidence of pathogenic infections that adversely affect tuber quality and yield. In recent years, several studies have focused on the microbiome diversity and biotechnological potential of endophytic microorganisms associated with potato plants. Finding new endophytic bacterial strains that can suppress potato pathogens and stimulate plant growth is an important challenge for researchers.

The objective of our study was to characterize the morphological and functional diversity of endophytic bacteria obtained in seed potato plants of five varieties.

2 Materials and methods

2.1 Materials

2.1.1 Plant material and sampling

Five cultivars of healthy potato (*Solanum tuberosum* L.) plants were used in this study: Reggi (Tatar Scientific Research Institute of Agriculture, Russia), Sprint (IJSSELMEER FOLDERS B.V., Netherlands), Gala (Norika, Germany), Red Sonia (Europlant, Germany), and Picasso (Agrico, Netherlands). The Reggi cultivar was analyzed in four subculture classes: Pre-basic field grown (PBFG), Super-super elite (S), Super elite (SE), and Elite (E). The remaining cultivars were obtained as Elite (E) class plants.

For each cultivar and class, five plants were collected at the flowering stage from the experimental fields of the Tatar Scientific Research Institute of Agriculture, Kazan, Russia (55°24′16″ N, 49°33′01″ E). Samples were transported to the laboratory under refrigerated conditions (4 °C) and processed within 48 h of collection.

2.2 Methods

2.2.1 Isolation of endophytic bacteria

Isolation of endophytic bacteria from plant leaves, stems, tubers, and roots of plants was carried out using the protocol provided by Anjum, Chandra [4]. To eliminate most of the epiphytic bacteria, individual parts of tubers, stems, leaves, and plant roots were rinsed in tap water and sequentially treated with 0.1% Tween 20 (for a few seconds), 70% ethanol (for 3 min), 5% NaOCl solution (for 1 min), 70% ethanol solution (for 2 min) and finally washed

three times with sterile distilled water. The top layer of each part was removed with a sterile scalpel and small portions of each plant organ (approximately 1-1.5 cm^2) were then plated on 2% sterile Luria-Bertani agar (LA) containing 60 µg/ml of nystatin to inhibit fungal growth. The plates were then incubated at 30°C for 48 hours. To ensure the sterility of the plant fragments, 100 µl of distilled water used for the final washing of the samples, was plated on sterile 2% LA and incubated at 30^0C. The lack of microbial growth on the nutrient medium after 48 hours of incubation, indicates the absence of epiphytic microflora on the surface of the plant fragments. Each separate colony of endophytic bacteria was repeatedly transferred to 2% LA plates and incubated for 24 hours at 30°C. Visual inspection of individual colonies grown on nutrient media and Gram staining of bacterial cells were used to assess the purity of the bacterial culture. All bacterial cultures were stored in glycerol at -70°C.

2.2.2 Morphological characterization of isolates

Colonies of pure cultures were characterized by the following features: color, size, form, surface, and appearance. Gram staining was performed for each pure culture to assess the cells shape and color. Isolates were cultured in a nutrient medium at 30°C for 48 hours to assess their ability to form endospores. Subsequently, preparations were made and stained using the Shaeffer-Fulton technique. The results of Gram staining and bacterial endospore staining were observed using oil immersion light microscopy (Micros MC 300 TXP, Austria).

2.2.2 Estimation of indole-3-acetic acid synthesis

All 49 endophytic isolates were estimated the abilities for IAA production. The bacterial cultures studied, obtained from the surface of 2% LA, were first transferred to LB medium and incubated at 37°C for 3 days. Cells were pelleted by centrifugation at 8,000 rpm for 5 min, and 1 ml of the supernatant was mixed with 4 ml of Salkowski reagent [5]. The reaction mixtures were incubated in the dark at room temperature for 20 min, and absorbance was measured at 530 nm using a spectrophotometer (Bio-Rad, USA). IAA concentrations were calculated from a standard curve of pure IAA ($y = 0.0147x + 0.1015$; $R^2 = 0.9717$).

2.2.3 Screening of endophytic isolates antagonistic activity

The antagonistic potential of the isolates was tested using a test culture of the phytopathogenic fungus *Fusarium oxysporum* DR57 (Department of Microbiology, Kazan (Volga region) Federal University, Russia).

The bacterial isolates were spread evenly on the surface of LB agar, and incubated at 30°C for 24 h. The fungal culture was cultivated using potato dextrose agar (PDA) at 30°C for 7-14 days. The agar blocks, containing the bacterial isolates and the fungal culture, were placed on the surface of the PDA medium, ensuring a distance of 4-5 cm between each block. The plates without bacterial isolates were used as control. The plates were incubated at a temperature of 30°C for a period of 7 to 14 days. Each experiment was conducted in triplicate to ensure reliability and accuracy of the results.

Antagonistic activity (AA) was determined by the percentage of phytopathogen growth inhibition according the formula:

$$AA\ (\%) = \left[\frac{\mathrm{K}-\mathrm{X}}{\mathrm{K}}\right] \mathrm{x}100,$$

where

X – diameter of the phytopathogen colony in the presence of endophytic bacterial isolate (mm);
K – diameter of the phytopathogen colony in control plate (mm).

2.2.4 MALDI-TOF mass spectrometry-based identification of endophytic bacteria

The MALDI Biotyper (Bruker Daltonics GmbH Germany) was used for identification of endophytic bacteria obtained. Bacterial isolates were incubated at 37°C for 24 h. Samples were prepared according to the method [6].

2.2.5 Statistical data analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA). Results are reported as mean ± SEM, and differences among means were considered statistically significant at $p < 0.05$.

For the partitioning of isolates based on different morphological characteristics matrix, IAA production, and antagonistic activity, hierarchical cluster analysis was performed using the Unweighted Average Linkage Clustering (UPGMA) algorithm. The clustering analysis utilized the Euclidean distance formula and k-means algorithms. The data analysis was conducted using R Core Team (2022). The results of the single-factor ANOVA (analysis of variance) confirmed that obtained mean values have statistically significant differences (having p-value lesser than conventional 0.05).

To assess the quality of clustering, appropriate visualizations and statistical tests were employed. Since the data did not follow a normal distribution, non-parametric tests were applied. Both the visualizations and statistical tests affirmed the presence of significant differences in means across various clusters.

3 Results

3.1 Isolation and characterization of morphological properties of endophytic bacteria

A total of 49 endophytic bacterial isolates were recovered from roots, tubers, stems, and leaves of five potato (*S. tuberosum* L.) cultivars originating from different regions: Reggi (Tatar Agriculture Research Institute, Russia), Sprint (IJSSELMEER FOLDERS B.V., Netherlands), Gala (Norika, Germany), Red Sonia (Europlant, Germany), and Picasso (Agrico, Netherlands). The Picasso variety yielded the highest number of endophytic bacteria isolates (10) among the different potato varieties (Fig. 1). Conversely, the Sprint variety yielded the lowest number of cultured endophytes (2).

Focusing on the *Reggi* variety, our analysis revealed that plants of the same cultivar but belonging to different propagation classes harbored distinct compositions of endophytic bacterial communities. Moreover, the early stages of seed potato propagation were characterized by the highest number of endophytic bacterial isolates (7) (Fig. 1). Subsequently, the number of endophytic bacteria decreased in seed potato plants of later breeding periods, starting from class SE.

Our investigation revealed that, in nearly all potato varieties examined, the majority of endophytic bacteria were localized within the root systems (Fig. 1). In contrast, the *Picasso* variety displayed a relatively uniform distribution of endophytes across different plant organs. Notably, endophytic isolates were also recovered from the tubers of the *Gala, Red Sonia*, and *Picasso* varieties.

In terms of morphological characteristics, the majority of isolates (81.6%) formed opaque colonies with a circular form (63.3%), cream color (67.3%), and a smooth (raised) surface (71.4%). Regarding size, more than half of the isolates (51%) displayed large colonies. The majority of the isolated bacterial strains (82%) exhibited a rod-shaped morphology. Gram staining revealed that 71% of the isolates were Gram-positive. Furthermore, 61% of the recovered isolates were spore-forming bacteria (Table 1).

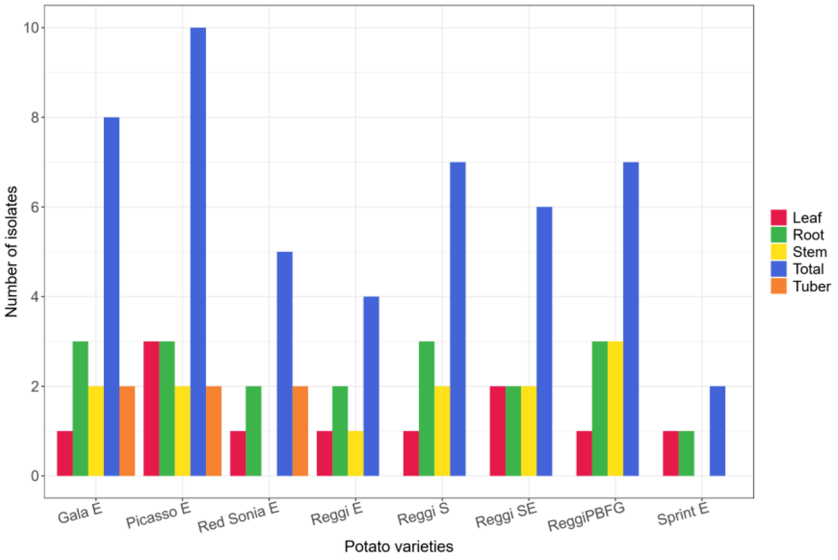

Fig. 1 The distribution of endophytic bacteria isolates obtained from the roots, stems, leaves, and tubers of five cultivars of seed potato plants. The abbreviations used in the figure represent different classes of potato plants: PBFG (pre-basic field grown), S (super-super elite), SE (super elite), and E (elite)

Table 1. Morphological and functional properties of endophytic bacteria isolated from seed potato plants

Isolates	**Colony morphology**					**Cell shape**	**Endospore formation**	**Gram reaction**	**IAA production, µg/ml**	**Antagonistic activity, %**
	Color	Form	Surface	Appearance	Size					
R PBFG R 10	creamy	circular	smooth	opaque	large	rod	+	+ ve	2.1±0.1	0
R PBFG R 11	creamy	circular	smooth	opaque	large	rod	+	+ ve	0	0
R PBFG R 12	gray	irregular	smooth	opaque	large	rod	_	- ve	0.7±0.05	0
R PBFG S 13	creamy	circular	smooth	opaque	medium	cocci	_	+ ve	0.7±0.03	0
R PBFG S 14	creamy	circular	wrinkled	opaque	large	rod	+	+ ve	0	14.2
R PBFG S 15	creamy	circular	smooth	opaque	large	rod	+	+ ve	1.9±0.01	21.3
R PBFG L 16	creamy	irregular	wrinkled	opaque	large	rod	+	+ ve	3.6±0.19	36.4
R S R 1	white	circular	hairy	opaque	large	rod	+	+ ve	2.8±0.11	40.8

R S R 2	creamy	rhizoid	wrinkled	opaque	large	rod	+	+ ve	0.1±0.01	0
R S R 3	creamy	circular	smooth	translucent	medium	rod	_	- ve	10.3±0.92	50.3
R S S 5	creamy	circular	smooth	translucent	medium	rod	_	- ve	1.9±0.02	0
R S S 6	creamy	circular	smooth	opaque	medium	cocci	_	+ ve	0.9±0.07	0
R S S 7	white	irregular	smooth	translucent	large	rod	+	+ ve	0	43.2
R S L 8	white	irregular	smooth	opaque	large	rod	+	+ ve	0.5±0.01	0
R SE R 17	creamy	circular	wrinkled	opaque	large	rod	+	+ ve	0.3±0.02	0
R SE R 18	gray	irregular	smooth	opaque	medium	rod	-	- ve	4.1±0.3	0
R SE S 19	creamy	irregular	wrinkled	opaque	large	rod	+	+ ve	1.8±0.01	0
R SE S 20	creamy	circular	wrinkled	opaque	large	rod	+	+ ve	3.6±0.32	0
R SE L 21	white	circular	smooth	opaque	medium	rod	-	+ ve	1.5±0.09	0
R SE L 22	white	circular	wrinkled	opaque	large	rod	+	+ ve	13.1±0.97	62.2
R E R 23	creamy	circular	hairy	opaque	large	rod	+	+ ve	1.1±0.01	38.7
R E R 24	creamy	rhizoid	wrinkled	opaque	small	rod	+	+ ve	1.5±0.12	0
R E S 25	creamy	circular	smooth	opaque	medium	cocci	-	+ ve	1.8±0.17	0
R E L 28	yellowish	irregular	smooth	opaque	medium	rod	+	+ ve	0	40.7
S R 134	creamy	irregular	smooth	opaque	large	rod	-	- ve	2.2±0.09	0
S L 129	white	irregular	smooth	translucent	medium	rod	+	- ve	3.7±0.28	0
G R 124	creamy	circular	smooth	opaque	small	rod	+	+ ve	3.3±0.31	0
G R 125	creamy	circular	smooth	opaque	medium	cocci	-	- ve	0.2±0.01	0
G R 126	creamy	irregular	smooth	opaque	large	cocci	-	- ve	3.8±0.25	0
G T 119	white	circular	smooth	opaque	large	rod	+	+ ve	2.3±0.21	45.3
G T 120	creamy	rhizoid	wrinked	opaque	large	rod	+	+ ve	1.7±0.15	0
G S 127	creamy	circular	smooth	translucent	medium	rod	-	- ve	4.9±0.42	0
G S 128	white	circular	smooth	translucent	medium	rod	-	- ve	4.3±0.39	0
G L 121	white	circular	smooth	opaque	large	rod	+	+ ve	0	0
RS R 115	creamy	irregular	smooth	translucent	medium	rod	-	- ve	5.4±0.45	43.5
RS R 116	creamy	circular	smooth	opaque	medium	cocci	-	+ ve	2.3±0.19	0
RS T 111	creamy	circular	smooth	opaque	large	rod	+	+ ve	3.7±0.25	42.8
RS T 112	creamy	rhizoid	wrinkled	opaque	small	rod	+	+ ve	0.7±0.04	48.3
RS S 114	creamy	irregular	smooth	translucent	medium	rod	-	- ve	2.6±0.19	0
P R 101	creamy	circular	smooth	opaque	medium	rod	+	+ ve	8.1±0.07	0
P R 102	white	circular	smooth	opaque	small	cocci	-	- ve	0	0
P R 109	creamy	circular	wrinkled	opaque	large	rod	+	+ ve	0	42.5
P T 105	creamy	circular	smooth	opaque	medium	cocci	-	+ ve	1.3±0.12	0

P T 106	white	circular	smooth	opaque	medium	cocci	-	- ve	0	0
P S 103	white	circular	smooth	opaque	medium	rod	+	+ ve	0.9±0.05	0
P S 104	creamy	circular	smooth	opaque	large	rod	+	+ ve	1.9±0.15	0
P L 107	white	circular	smooth	translucent	medium	rod	+	+ ve	1.2±0.10	0
P L 113	creamy	circular	smooth	opaque	large	rod	+	+ ve	2.3±0.21	37.7
P L 122	creamy	rhizoid	wrinkled	opaque	large	rod	+	+ ve	11.0±0.97	0

Legend: potato cultivars: R – Reggi, S – Sprint, G – Gala RS – Red Sonia, P – Picasso; Classes of potato: PBFG – pre-basic field grown, S – super-super elite, SE – super elite, E – elite; organs of potato plants: R – root, L –leaf, S – steam, T –tuber. Positive reaction (+); Negative reaction (–); + ve – Gram positive and – ve – Gram negative.

3.2 IAA production

The data presented in Table 1 indicate that a significant part of the isolates (83.7%), were able to produce IAA. The highest amounts of IAA were detected in the culture medium of isolates R SE L 22 (13.1 µg/ml), R S R 3 (10.3 µg/ml), and P L 122 (11 µg/ml). These isolates were obtained from the leaves and roots of Reggi variety plants (super elite and super-super elite classes), and from the leaves of the Picasso variety, respectively (Table 1).

3.3 Screening of the antagonistic activity of endophytic isolates

Antagonistic potential of 49 endophytic isolates obtained from different organs of potato plants against the phytopathogenic strain *F. oxysporum* DR57 was defined. Fifteen of the 49 isolates (30.6%) were found to have the ability to inhibit the growth of *F. oxysporum* DR57. The antagonistic activity of these isolates ranged from 14.2% to 62.2% (Table 1). In particular, three isolates R SE L 22, R S R 3, and RS T 112, showed significant inhibition rates, suppressing the growth of *F. oxysporum* DR57 by 62.2%, 50.3%, and 48.3%, respectively (Table 1). So, the results obtained suggest that these isolates can be considered as promising candidates for biological control of diseases caused by *F. oxysporum*, one of the most common potato pathogens.

3.4 Cluster analysis of the morphological and functional characteristics of endophytic isolates

The UPGMA algorithm was initially used to cluster the isolates, resulting in the formation of four clusters. However, the clustering was unbalanced, with the second cluster containing only one observation and the third cluster containing almost 90% of the data. To achieve a more balanced and informative clustering, we then applied the k-means algorithm. The k-means clustering algorithm effectively divided the 49 endophytic isolates into six distinct clusters (Fig. 2).

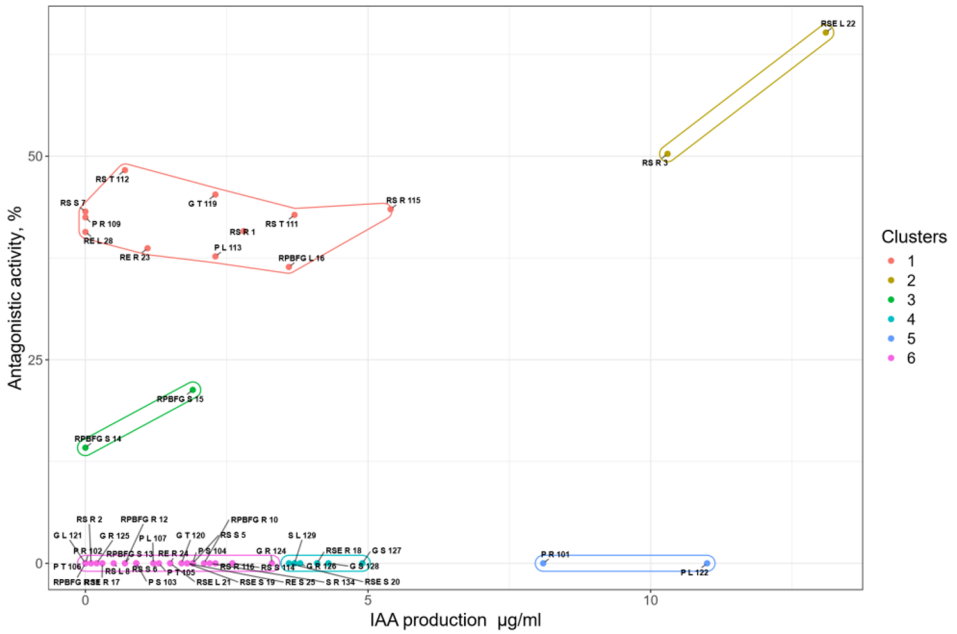

Fig. 2 A scatterplot showing the results of the k-means clustering of endophytic bacteria isolates obtained from seed potato plants. The clusters are indicated by different colors, and boundary lines separate the clusters. Potato cultivars: R – Reggi, S – Sprint, G – Gala RS – Red Sonia, P – Picasso; Classes of potato: PBFG – pre-basic field grown, S – super-super elite, SE – super elite, E – elite; organs of potato plants: R – root, L –leaf, S – steam, T –tuber.

The effectiveness of the k-means clustering is supported by the visualisation and the statistical tests, which indicate the presence of statistically significant differences in the means between the six clusters. The p-values obtained from the statistical tests are remarkably low, allowing us to reject the null hypothesis and conclude that the means of the different clusters are not equal to each other. To further enhance the visualisation of the k-means clustering results, we generated a boxplot graph (Fig. 3). The boxplot shows that the medians, as well as the 0.25 and 0.75 quantiles, for IAA production differ in most clusters. Meanwhile for antagonistic activity, only the clusters 1, 2 and 3 show values above zero, while the other clusters show antagonistic activity equal to zero (Fig. 3). In particular, cluster 2 contains two isolates, R SE L 22 and R S R 3, which are characterised by the highest values for both IAA production and antagonistic activity (Fig. 2).

Cluster 6 contains the largest number of isolates (26), but these isolates have relatively low IAA production and antagonistic activity. Cluster 1 consists of 11 isolates, the majority of which are endospore-forming bacteria. Gram-positive bacteria predominate in clusters 1, 3, 5, and 6, while cluster 4 consists mainly of Gram-negative bacteria.

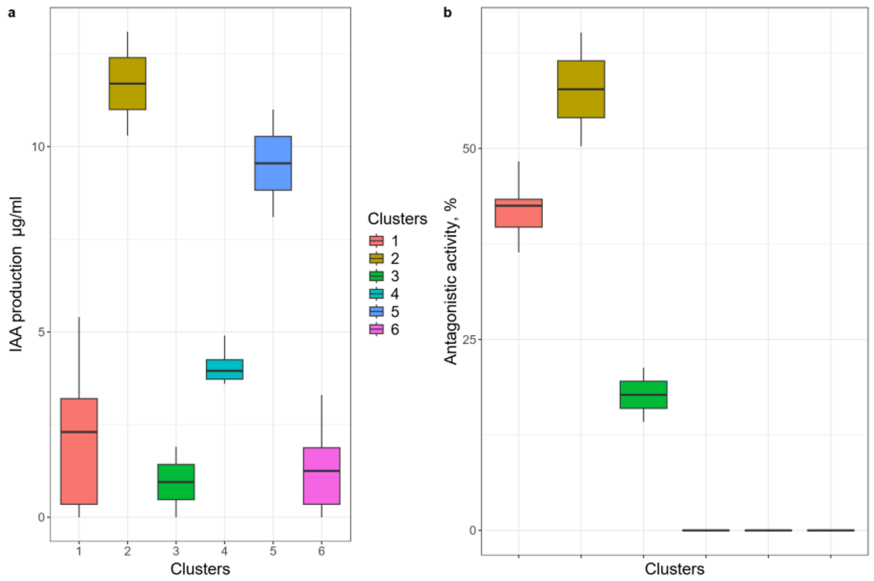

Fig. 3 Boxplots showing the distribution of IAA production (a) and antagonistic activity (b) among the six clusters of endophytic bacterial isolates. The bold line inside each box represents the median of the data, while the bottom of the box corresponds to the 0.25 quantile and the top to the 0.75 quantile.

3.5 Identification of endophytic bacteria isolates by MALDI-TOF analysis

The identification results obtained by MALDI-TOF MS are presented in the Table 2. The analysis revealed significant variation in the composition of endophytic bacterial communities among the studied cultivars, including cultivar Reggi. According to the data obtained, the isolated endophytes were taxonomically classified into several genera: ***Bacillus, Lysinibacillus, Acinetobacter, Staphylococcus, Enterobacter***. It should be emphasized that the majority of isolates (59%) belong to the genus *Bacillus*.

Table 2. Endophytic bacterial isolates identified by MALDI TOF-MS

Isolates	Bacterial species	Cultivars and classes of potato*
R PBFG R 10	*Bacillus megaterium*	Reggi PBFG
R PBFG R 11	*Bacillus megaterium*	
R PBFG R 12	*Enterobacter cloacae*	
R PBFG S 13	*Staphylococcus warneri*	
R PBFG S 14	*Bacillus pumilus*	
R PBFG S 15	*Bacillus subtilis*	
R PBFG L 16	*Bacillus pumilus*	
R S R 1	*Bacillus mycoides*	Reggi S
R S R 2	*Bacillus pumilus*	
R S R 3	*Enterobacter ludwigii*	
R S S 5	*Enterobacter ludwigii*	

R S S 6	*Staphylococcus warneri*	
R S S 7	*Bacillus subtilis*	
R S L 8	*Bacillus simplex*	
R SE R 17	*Bacillus pumilus*	Reggi SE
R SE R 18	*Enterobacter ludwigii*	
R SE S 19	*Bacillus pumilus*	
R SE S 20	*Bacillus pumilus*	
R SE L 21	*Lysinibacillus fusiformis*	
R SE L 22	*Bacillus toyonensis*	
R E R 23	*Lysinibacillus fusiformis*	Reggi E
R E R 24	*Bacillus weithenstephanensis*	
R E S 25	*Staphylococcus warneri*	
R E L 28	*Bacillus horneckiae*	
S E R 134	*Enterobacter cloacae*	Sprint E
S E L 129	*Bacillus mojavensis*	
G E R 124	*Bacillus mojavensis*	Gala E
G E R 125	*Enterobacter hormaechei*	
G E R 126	*Bacillus cereus*	
G E T 119	*Bacillus cereus*	
G E T 120	*Bacillus pumilus*	
G E S 127	*Enterobacter ludwigii*	
G E S 128	*Enterobacter ludwigii*	
G E L 121	*Bacillus megaterium*	
RS E R 115	*Enterobacter ludwigii*	Red Sonia E
RS E R 116	*Staphylococcus warneri*	
RS E T 111	*Bacillus cereus*	
RS E T 112	*Bacillus weithenstephanensis*	
RS E S 114	*Enterobacter ludwigii*	
P E R 101	*Bacillus subtilis*	Picasso E
P E R 102	*Acinetobacter pittii*	
P E R 109	*Bacillus thuringiensis*	
P E T 105	*Staphylococcus warneri*	
P E T 106	*Acinetobacter baumannii*	
P E S 103	*Bacillus mojavensis*	
P E S 104	*Bacillus subtilis*	
P E L 107	*Bacillus subtilis*	
P E L 113	*Bacillus subtilis*	
P E L 122	*Bacillus pumilus*	

Legend: potato cultivars: R – Reggi, S – Sprint, G – Gala RS – Red Sonia, P – Picasso; Classes of potato: PBFG – pre-basic field grown, S – super-super elite, SE – super elite, E – elite; organs of potato plants: R – root, L –leaf, S – steam, T –tuber.

4 Discussion

Numerous studies have shown that endophytes maintain a friendly relationship with their hosts and contribute to improving of plant vitality. Therefore, research aimed at assessing the biodiversity and functional role of endophytic microorganisms is highly relevant.

We have shown that potato plants representing five varieties grown under identical environmental conditions have different quantitative compositions of endophytic bacteria. The highest number of endophytic bacterial isolates (10) was recovered from the *Picasso* variety, whereas the *Sprint* variety yielded the lowest number of isolates (2). (Fig. 1). The colonization of plants by endophytic microorganisms depends on many factors, including the genotype of the plant, the type of plant tissue, the species of microorganisms, other biotic and abiotic factors. Successful invasion of endophytes depends on coordinated signalling systems for communication between plants and microorganisms, as well as within the endophytic population]. It is well known that, genetic changes resulting from breeding selection can lead to changes in the endophytic community between different varieties of the same plant species [7].

It should be emphasized that, according to the data obtained, potato plants of different classes of the *Reggi* variety are also characterized by different quantitative composition of endophytic bacteria. Thus, plants classified as Pre-Basic Field-Grown exhibited the greatest abundance of endophytic bacterial isolates. It can be assumed that endophytic bacteria provide additional protection to plants during the first years of life. It is possible that as the plants age, they become more selective of endophytes, favouring the most beneficial microorganisms from the initial pool.

The highest abundance of endophytic bacteria was detected in the root systems. Endophytes were isolated from tubers only in three varieties – *Gala*, *Red Sonia*, and *Picasso* (Fig. 1). This observation may indicate that these cultivars exhibit a relatively rapid vascular system development, facilitating the systemic translocation of endophytes to aerial and subterranean organs, including the tubers.

The quantitative distribution of endophytic bacteria across different plant organs aligns with previous reports [8]. The elevated abundance of endophytes in roots is likely attributable to the active secretion of root exudates, which serve as nutrient sources for microorganisms.

The morphological characteristics of the endophytic isolates provided us with initial data on the diversity of the endophytic microbiota of the potato plants studied. The data obtained showed that the dominant species among the cultivated endophytes are bacteria that form opaque, circular-shaped colonies with a smooth surface and cream color. The predominant cell shape is rod-shaped. It should be emphasised that the majority of endophytic isolates strains belong to Gram-positive bacteria and have the ability to form endospores (Table 1). It is known that many of the Gram-positive bacteria are known to synthesise unique metabolites that can stimulate plant growth, protect their hosts from abiotic stress factors, control pathogenic microorganisms, and improve plant health [9].

The identification of endophytic bacteria isolated from potato plants using MALDI-TOF MS method showed that a total of 49 endophytic bacteria covering 5 genera (*Lysinibacillus, Staphylococcus, Enterobacter, Acinetobacter, Bacillus*) and 18 species were identified (Table 2). The majority of isolates (59%) belonged to the genus *Bacillus*, most abundant species being *Bacillus pumilus*.

The results obtained regarding the dominance of bacteria belonging to the genus *Bacillus* in the endophytic communities of potato plants are consistent with many other reports.

Endophytic bacteria from the genus *Bacillus* are known for their production of various physiologically active compounds [10]. It can also be hypothesized that bacteria capable of producing endospores have more opportunities to live in the endosphere of the host and to maintain viability over the multiple generations of the same plant.

The results of our study regarding the ability of the majority of potato endophytic isolates (83.7%) to produce IAA confirm the involvement of these bacteria in key physiological processes of their hosts and highlight the importance of IAA as a "communication molecule" between endophytes and plants. Tthe highest level of IAA production demonstrated isolates R SE L 22 (13.1 μg/mL) and R S R 3 (10.3 μg/mL), obtained from the Reggi potato cultivar of the super-elite and super-super elite classes, respectively (Table 1, Fig. 2).

Previous studies have demonstrated the ability of potato endophytic bacteria to synthesize IAA. Thus, the presence of the endophytic strain *Methylobacterium* sp. 2AB in potato correlated with the intense root hairs development suggesting a role of IAA synthesis in plant growth [11].

Infectious diseases caused by fungal pathogens are a serious threat to potato production and quality. Among the most widespread and dangerous are potato diseases caused by *Fusarium* species, which manifest themselves as vascular wilt, root rots and rot of tubers, bulbs and corms [12].

We have shown that 15 of the 49 potato endophytic isolates have antagonistic potential against the phytopathogenic strain *F. oxysporum* DR 57. The most profound effect was observed for isolates R SE L 22 (65.2%), R S R 3 (50.3%), and RS T 112 (48.3%) (Table 1, Fig. 3). Our results are in agreement those of other researchers who have demonstrated the antagonistic activity of potato endophytic microorganisms against different *Fusarium* species. For example, Pageni *et al*. reported the antagonistic effect of bacterial endophytes isolated from potato roots against *F. sambucinum* [13]. Treatment of potato tubers with endophytic *Bacillus subtilis* 10-4 and *B. subtilis* 26D strains reduced the incidence of dry rot infections caused by *F. oxysporum* up to 50% [14]. These results highlight the potential of endophytic microorganisms for biocontrol of potato fungal phytopathogens.

The use of hierarchical cluster analysis by UPGMA and k-means clustering methods on the morphological and functional characteristics of 49 endophytic isolates allowed for a clear demonstration the diversity of endophytic bacteria community in different potato varieties (Fig. 2 and 3). Moreover, it was established that the most interesting cluster is cluster 2, which contains promising isolates with the highest production values of the phytohormone IAA and antagonistic activity against the potato pathogen *F. oxysporum* DR 57.

5 Conclusion

Understanding the role of endophytic microorganisms in potato plants is crucial for the development of sustainable and environmentally friendly strategies to enhance the yields of this important crop. This study represents the first comprehensive analysis of cultured endophytic bacterial community in the potato cultivars Reggi, Sprint, Gala, Red Sonia, and Picasso. Our results indicate that the amount and species composition of endophytic bacteria vary between different potato cultivars and classes. Notably, the majority of the endophytic isolates were Gram-positive bacteria, primarily from the genus *Bacillus*. A significant part of total 49 endophytic isolates demonstrated the ability to synthesize the phytohormone indole-3-acetic acid and antagonistic activity against strain *Fusarium oxysporum* DR57, emphasizing their functional importance for potato plants. The results of the study provide insights into the diversity and functional properties of endophytic microorganisms in potato plants and their impact on plant health and productivity.

Acknowledgments

This study was supported by the Russian Science Foundation (RSF) grant №25-16-00143.

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