

## Article

# What Bacteria Are Present in the Endosphere of Lettuce Seeds and Why?

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## Abstract

Increasing demand for high-quality food is driving the development of biologized farming methods, which involve the use of microorganisms, including endophytes, to stimulate plant growth. However, research on the composition of endosphere microbiomes is limited. The study presents an analysis of the bacterial endophytic microbiome in lettuce seeds (*Lactuca sativa* L., cv. Ozornik) using high-throughput sequencing of 16S rRNA amplicons. It evaluates the taxonomic composition and putative functional properties of seed endophytic bacteria. The microbial community exhibited low diversity (Shannon index ranged from 1.1 to 1.84, Simpson index from 0.57 to 0.83). The bacterial endophytic community of lettuce seeds was dominated by Pseudomonadota (83%), Actinomycetota (14%), and Bacillota (3%). The genera identified within the microbiome included Pantoea (32%), Rhodococcus (13%), Candidatus Profftella (13%), Janthinobacterium (7%), Pseudomonas (9%), Enterococcus (3%), and Alcaligenes (2%), which exhibit a broad spectrum of beneficial properties: plant growth promotion (PGPB), suppression of phytopathogens, enhanced stress tolerance, participation in contaminant biodegradation, and heavy metal detoxification. The structure and functional potential of the microbiome vary between samples, potentially due to differences in source material and cultivation conditions. The obtained results expand our understanding of the composition and functions of endophytic bacteria in lettuce seeds, which is important for the development of novel biocontrol agents for plants consumed by humans in an unprocessed form.

**Keywords:** seed endophytes; lettuce; endophytic bacterial microbiome; endophytic bacteria



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## 1. Introduction

The seed endophytic microbiome refers to communities of microorganisms, predominantly bacteria and fungi, residing within the internal tissues of seeds without causing disease [1]. These microbiomes differ from those colonizing the external surface of seeds (epiphytes) and are characterized by a close association with the embryo and endosperm, which facilitates vertical transmission (from parent to offspring) of the microbiota across plant generations [2,3]. Seed endophytes are more likely to be inherited by subsequent generations, potentially forming the initial microbial community of the seedlings.

Seed endophytic microbiomes often include representatives from various taxa such as Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes. Among the most frequently

identified genera are *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Micrococcus*, *Acinetobacter*, and *Staphylococcus* [2,4]. The seed microbiome plays a crucial role in shaping plant health, influencing growth, resistance to pathogens, and adaptation to environmental conditions [5,6]. Endophytic microbes are often the primary colonizers of germinating seedlings, contributing to the early establishment of the microbiome and protecting seedlings from pathogenic microbes through competitive exclusion or colonization [1,2,7,8]. Many endophytes produce phytohormones, such as indole-3-acetic acid, secrete enzymes like ACC deaminase (which reduces plant stress hormone levels), and assist in breaking seed dormancy, thereby directly enhancing seed viability, germination rate, and seedling vigor [2,7,9]. Seed endophytes facilitate nutrient uptake by plants through atmospheric nitrogen fixation, phosphorus mobilization, siderophore production (iron chelators), and secretion of organic acids that solubilize minerals from the surrounding environment, providing competitive advantages under nutrient-limited conditions [10]. Seed endophytes also help plants cope with abiotic stresses—such as drought, salinity, and extreme temperatures—by modulating host physiology and activating systemic resistance mechanisms. These enhancements in stress tolerance and adaptive responses are particularly critical during the early stages of plant development, when seedlings are most vulnerable [2,9]. The application of beneficial seed endophytes via seed treatment or coating is being investigated as an alternative to chemical seed treatments, offering promising avenues for sustainable plant protection and yield improvement.

In recent years, increasing attention has been paid to the composition and functions of microbial communities associated with the seeds of agricultural crops [11]. The use of biocontrol agents containing seed endophytes for seed pretreatment is considered an alternative to chemical treatments, offering opportunities for sustainable plant protection and yield enhancement while simultaneously reducing the environmental pesticide burden. Lettuce (*Lactuca sativa* L.) is a widely consumed vegetable, often eaten raw, which increases the risk of foodborne diseases in humans [12,13]. Accordingly, the microbiome of *Lactuca sativa* L. seeds is attracting growing interest due to its potential impact on plant health and the safety of products consumed unprocessed by humans. Seeds serve not only as a reservoir for the transmission of microorganisms to the next plant generation, but also as a potential source of beneficial microbes capable of enhancing crop productivity and stress tolerance [14,15]. Despite the growing interest in phytomicrobiomes, the composition and functional significance of the lettuce seed microbiome remain poorly understood [16]. Low microbial biomass and diversity in seeds complicate cultivation-independent studies and often lead to pooled analyses that obscure intraspecific variability within seeds [1]. The functional roles of individual seed-transmitted endophytes remain poorly understood, particularly regarding their contributions to the host's adaptability to various environmental conditions [17,18]. Modern molecular genetic techniques, such as high-throughput sequencing of 16S rRNA and ITS regions, enable detailed analysis of microbial diversity, identification of dominant bacterial and fungal taxa, and assessment of their potential roles in early plant development [19]. However, the principles governing the formation of endophytic microbial communities, the transmission pathways, and the ecological interactions between seed endophytes and phytopathogens in crops such as lettuce remain insufficiently understood. Studying the lettuce seed microbiome is not only of theoretical interest, but also of practical importance for the development of biological products based on beneficial microorganisms that enhance seed germination and plant resistance to biotic and abiotic stresses [20,21].

The aim of this study is a comprehensive analysis of the microbial community of *L. sativa* seeds using modern metagenomic methods to identify key taxa that may influence plant growth and development and, subsequently, they can be used as environmentally

friendly biological control agents. Such biocontrol agents reduce reliance on chemical pesticides and enhance food safety. Additionally, the integration of seed microbiome knowledge into breeding and production processes can facilitate the development of crop varieties with improved pathogen resistance, constituting a part of a holistic approach to plant health management.

## 2. Materials and Methods

### 2.1. Seed Preparation

For the experiment, lettuce seeds (*Lactuca sativa* var. *crispa* L.) of the Ozornik variety, produced by Agrofirma AELITA LLC, Moscow, Russia, were selected. The seeds were produced in 2023 and packaged in 2024. For the study, seeds from a single lot were used to exclude heterogeneity caused by different seed production conditions. To assess the endophytic bacterial microbiome of the seeds, surface sterilization was performed. Specifically, lettuce seeds were treated with 2.5% sodium hypochlorite for 2 min, rinsed with sterile tap water, and then immersed in 70% ethanol for 3 min. The sterilized seeds were thoroughly washed five times with sterile tap water. The final rinse water was plated onto Petri dishes containing LB agar. In the absence of bacterial or fungal colony growth, the seed sterilization process was considered successful, and such seeds were used for subsequent DNA extraction from the endosphere microbiome [22].

### 2.2. DNA Extraction, Amplicon Library Preparation and Sequencing

The MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) was used for DNA extraction according to the manufacturer's instructions. DNA concentration and quality were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). The bacterial community was sequenced using Illumina technology (Illumina, San Diego, CA, USA). Genomic library preparation was performed following the 16S metagenomic sequencing library preparation protocol (Illumina MiSeq, San Diego, CA, USA). The V3–V4 region of the 16S rRNA gene was amplified using a DNA Engine Tetrad<sup>®</sup> 2 thermal cycler (Bio-Rad, Hercules, CA, USA) with the following primers: Illumina\_16S\_341F (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCC-TACGGGNGGCWGCAG) and Illumina\_16S\_805R (GTCTCGTGGGCTCGGAGATGTG-TATAAGAGACAGGACTACHVGGGTATCTAATCC). Amplification conditions were as follows: initial denaturation at 95 °C for 3 min; 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; followed by a final elongation at 72 °C for 3 min. Amplicons were purified using the Agencourt AMPure XP kit (Beckman Coulter, Brea, CA, USA). A second round of amplification was performed under the same conditions. Amplicon concentration was measured with a Qubit 3.0 fluorometer (Invitrogen, Carlsbad, CA, USA) using the Quant-iT<sup>™</sup> high-sensitivity DNA assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Quality control of the amplicons was conducted on a LabChip GX Touch 24 system (PerkinElmer, Waltham, MA, USA). Sequencing was performed on a MiSeq instrument (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The 16S rRNA sequencing data were analyzed using the DADA2 algorithm [23]. Raw paired-end reads were processed with the filterAndTrim function from the DADA2 pipeline to remove low-quality sequences and adapter contamination. Forward (R1) and reverse (R2) reads were trimmed to fixed lengths of 240 and 225 base pairs, respectively, to eliminate low-quality regions at the 3' ends. Sequences containing ambiguous bases (N) were discarded (maxN = 0), and reads with an expected error rate higher than 2 (maxEE = c(2, 2)) were filtered out. To correct for sequencing errors, error profiles for forward and reverse reads were independently estimated using the learnErrors function. The accuracy of the error models was visually assessed with plotErrors, by comparing observed error rates to the

expected quality scores (nominalQ = TRUE). Subsequently, the dada function was applied for sample-specific error correction and denoising, generating exact amplicon sequence variants (ASVs). Forward and reverse reads were merged using mergePairs. Merged sequences were compiled into an ASV abundance table using makeSequenceTable (DADA2 version 1.34.0), and the length distribution of sequences was examined. Potential chimeric sequences were identified and removed via the removeBimeraDenovo function with the consensus method, ensuring retention of only biologically valid ASVs. Finally, taxonomic classification was performed using the SILVA v138.2 reference database through the assign-Taxonomy function. Prior to downstream analyses, all ASVs identified as “chloroplast” or “mitochondria” were removed.

### 2.3. Statistical Analysis

Further statistical analysis of the relative abundance data of amplicon sequence variants was performed using the free R software environment (version 4.4.3). Alpha diversity of bacterial communities was assessed using the Shannon and Simpson indices via the diversity function of the vegan package.

## 3. Results

### *Taxonomic Composition of the Endophytic Bacterial Community of Lettuce Seeds*

Metagenomic sequencing of amplicons yielded 65,377, 73,471, and 60,108 raw reads for the 16S rRNA gene in the respective samples. After quality filtering using the filterAndTrim function, 40,515, 46,073, and 36,096 reads were retained, respectively. The DADA2 algorithm identified 6006, 6509, and 5604 unique sequences in the samples. It is noteworthy that all samples contained a high proportion of reads identified as lettuce chloroplast (39%) and mitochondrial (43%) sequences, which were removed prior to analysis. A total of 18 amplicon sequence variants (ASVs) were obtained across all samples after processing. Taxonomic composition of the bacterial endophytic bacterial microbiome of lettuce seeds is presented in Table 1. The bacterial community of the lettuce seed endosphere was dominated by representatives of the phylum Pseudomonadota (83% relative abundance), Actinomycetota (14%), and Bacillota (3%). The phylum Pseudomonadota was represented by the class Gammaproteobacteria, which included the orders Enterobacterales, Burkholderiales, and Pseudomonadales. The phylum Actinomycetota was represented by the class Actinobacteria, comprising the order Mycobacteriales. The phylum Bacillota was represented by the class Bacilli, including the order Lactobacillales. The endospheric microbial community of lettuce seeds included representatives of the genera *Pantoea* (32%), *Rhodococcus* (13%), *Candidatus Profftella* (13%), *Janthinobacterium* (7%), *Pseudomonas* (9%), *Enterococcus* (3%), and *Alcaligenes* (2%). The Shannon diversity indices for the three samples were 1.84, 1.51, and 1.1, respectively. The Simpson indices were 0.83, 0.76, and 0.57 for the three samples.

**Table 1.** Taxonomic composition of the bacterial endophytic bacterial microbiome of lettuce seeds (*Lactuca sativa* L.) revealed by 16S rRNA gene amplicon sequencing. Relative abundance is indicated as a percentage (%). Values are presented for three independent samples (C1–C3). Taxa identified as “chloroplast” or “mitochondria” are not included. NA—data not identified.

Kingdom	Phylum	Class	Order	Family	Genus	16S-C1_S252	16S-C2_S253	16S-C3_S254
Bacteria	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Erwinaceae	Pantoea	0.00	0.00	59.52
Bacteria	Actinomycetota	Actinobacteria	Mycobacteriales	Nocardiaceae	Rhodococcus	0.00	0.00	26.19
Bacteria	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Erwinaceae	Pantoea	0.00	36.00	0.00

Table 1. Cont.

Kingdom	Phylum	Class	Order	Family	Genus	16S-C1_S252	16S-C2_S253	16S-C3_S254
Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	Candidatus Proffttella	23.08	0.00	0.00
Bacteria	Pseudomonadota	Gammaproteobacteria	Enterobacterales	NA	NA	23.08	0.00	0.00
Bacteria	Pseudomonadota	Gammaproteobacteria	NA	NA	NA	19.23	0.00	0.00
Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	0.00	20.00	0.00
Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.00	20.00	0.00
Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	Candidatus Proffttella	0.00	16.00	0.00
Bacteria	Actinomycetota	Actinobacteria	Mycobacteriales	Nocardiaceae	Rhodococcus	11.54	0.00	0.00
Bacteria	Cyanobacteriota	Cyanobacteriia	Chloroplast	NA	NA	0.00	0.00	0.00
Bacteria	Pseudomonadota	Gammaproteobacteria	Enterobacterales	NA	NA	7.69	0.00	0.00
Bacteria	Bacillota	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	7.69	0.00	0.00
Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	7.69	0.00	0.00
Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	0.00	8.00	0.00
Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Alcaligenaceae	Alcaligenes	0.00	0.00	4.76
Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0.00	0.00	4.76
Bacteria	Actinomycetota	Actinobacteria	NA	NA	NA	0.00	0.00	4.76

#### 4. Discussion

At the next stage, the beneficial properties of lettuce seed endophytes were analyzed (Table 2). Bacteria of the genus *Pantoea* are common components of the seed microbiome and perform several key functions that promote seed germination and plant growth. In particular, numerous studies have demonstrated that *Pantoea* spp. are plant growth-promoting bacteria (PGPB) due to their ability to produce phytohormones such as auxins, gibberellins, and cytokinins, which stimulate root system development and seed germination. They also synthesize siderophores that enhance iron availability for the plant and fix atmospheric nitrogen, thereby improving soil nutritional value [21,24,25].

Furthermore, some strains of *Pantoea* exhibit antagonistic activity against phytopathogenic fungi and bacteria by producing antimicrobial compounds (e.g., pantocin and herbicides) and through strong competition for nutrients and colonization niches [16,26]. The synthesis of osmoprotectants, such as proline, enables increased plant tolerance to abiotic stresses including drought and salinity [19]. Additionally, *Pantoea* has been reported to enhance plant biotic resistance via the induction of systemic resistance (ISR) [20]. Studies have shown that *Pantoea agglomerans* and related species can accelerate lettuce seed germination by degrading growth inhibitors in the seed coat and activating metabolic processes within the embryo [14,15].

**Table 2.** Beneficial properties of endophytic bacterial microbiome. Reported functions from the literature include the ability to stimulate plant growth (PGPB), suppress phytopathogens, enhance tolerance to abiotic stresses, accelerate seed germination, and detoxify pollutants. Symbols: ‘+’—confirmed activity; empty cells—no data available. Percentages in parentheses represent the average relative abundance of each genus in the microbiome.

		<i>Pantoea</i> (32%)	<i>Rhodococcus</i> (13%)	<i>Enterococcus</i> (3%)	<i>Alcaligenes</i> (2%)	<i>Janthinobacterium</i> (7%)	<i>Pseudomonas</i> (9%)
	phytohormones	auxins, gibberellins, cytokinins	IAA	auxins, cytokinins	IAA, gibberellins	IAA	IAA, gibberellins
PGPB	siderophores	+		+	+	+	pyoverdine
	nitrogen fixation	+			+		+
	phosphorus mobilization		+	+	+	+	+
Suppression of phytopathogens	antimicrobial compounds	pantocin and herbicides	+	enterocins	+	violacein, quinazoline alkaloids	2,4-diacetyl-loroglucine (DAPG), phenazines (e.g., phenazine-1-carboxylic acid), pyoluteorin
	competition	+	+	+			
	induction of systemic resistance (ISR)		+				
Increased stress tolerance	cold					cryoprotective proteins	
	drought	+					
	salinization	proline				proline, glycine betaine	proline, trehalose
	oxidative stress	proline		osmoprotectants		proline, glycine betaine	proline, trehalose
Seed germination		decomposition of growth inhibitors in the seed coat, activation of metabolic processes in the embryo					
Detoxification			destruction of pesticides, PAHs and alkanes	decomposition of phenols and organochlorine compounds	detoxification of heavy metals		

Bacteria of the genus *Rhodococcus* are important components of seed microbiomes and can perform several key functions that promote seed germination and enhance plant resistance. Their role in lettuce seeds has been studied less extensively than that of other endophytes; however, research on other crops suggests the following possible functions. First, *Rhodococcus* spp. are known for their ability to degrade a wide range of organic pollutants, including pesticides, polycyclic aromatic hydrocarbons (PAHs), and alkanes, owing to their powerful catabolic enzymes (such as cytochrome P450 and dioxygenases) [27,28]. Some strains of *Rhodococcus* are considered plant growth-promoting bacteria (PGPB) due to their synthesis of phytohormones (e.g., indole-3-acetic acid, IAA) and their capacity to solubilize phosphates [29]. Additionally, *Rhodococcus* strains can suppress pathogens through quorum sensing interference, VOC synthesis, competitive exclusion, direct antagonism (via the



production of antimicrobial compounds), and the induction of systemic resistance (ISR) in plants [30,31]. *Rhodococcus* is frequently detected in seeds, but its mechanisms of vertical transmission and ecological roles require further investigation [14,32].

The genus *Candidatus Proffttella* represents a poorly understood group of bacteria classified as insect endosymbionts, and their potential role in plant seeds, including those of lettuce, warrants special consideration. To date, no direct studies have confirmed the presence or functional activity of *Ca. Proffttella* in lettuce seeds. This genus is a highly specialized insect endosymbiont with a well-established ecological niche and defined functions within the *Diaphorina citri* system, which is associated with citrus pathogens [33].

Bacteria of the genus *Janthinobacterium* are recognized as multifunctional endophytes capable of influencing plant growth and enhancing stress resistance. *Janthinobacterium* spp. produce several antimicrobial compounds, including violacein—a pigment with demonstrated activity against fungi (e.g., *Fusarium*, *Pythium*) and pathogenic bacteria—as well as quinazoline alkaloids that inhibit the growth of competing microorganisms [34].

Furthermore, *Janthinobacterium* species are classified as plant growth-promoting bacteria (PGPB) due to their synthesis of indole-3-acetic acid (IAA), phosphate solubilization, and siderophore production [35]. The induction of osmoprotectants (such as proline and glycine betaine) and the synthesis of cryoprotective proteins by *Janthinobacterium* strains contribute to increased bacterial resistance to drought and cold stress [36].

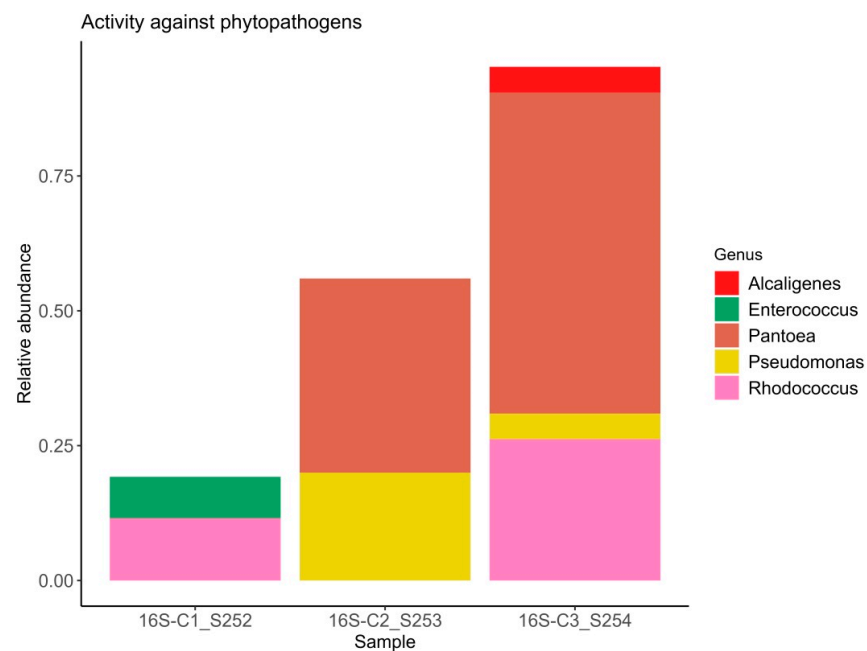
Bacteria of the genus *Pseudomonas* (particularly the species *P. fluorescens*, *P. putida*, and *P. chlororaphis*) are key components of the seed microbiome of lettuce and perform numerous functions critically important for plant germination, growth, and resistance. First, *Pseudomonas* spp. are known to suppress the growth and development of phytopathogens through the synthesis of antimicrobial metabolites such as 2,4-diacetylphloroglucinol (DAPG), phenazines, and pyoluteorin, competition for iron via siderophore production (e.g., pyoverdine), and induction of systemic resistance (ISR) in plants [37]. It has been demonstrated that *P. fluorescens* suppresses *Fusarium* by synthesizing siderophores [38], while phenazines produced by *P. chlororaphis* protect lettuce seeds from *Pythium* [39]. *Pseudomonas* species are classified as plant growth-promoting bacteria (PGPB) due to their ability to synthesize phytohormones (indole-3-acetic acid (IAA), gibberellins) [40], solubilize phosphates [41], and some strains can fix atmospheric nitrogen [42]. Moreover, *Pseudomonas* spp. have been shown to alleviate salt stress in lettuce by regulating ionic homeostasis [43], and *Pseudomonas putida* enhances lettuce resistance to drought by modulating phytohormones and antioxidants [44]. *Pseudomonas* actively colonizes seeds owing to its ability to form biofilms (facilitating adhesion to the seed coat) and to undergo horizontal transmission through soil and water [14,45].

Bacteria of the genus *Enterococcus* are rarely considered typical components of the phytomicrobiome, but their presence in lettuce seeds may be associated with several ecological and functional roles. Some *Enterococcus* strains exhibit plant growth-promoting properties (PGPB) through the synthesis of phytohormones (auxins, cytokinins), phosphate solubilization, and siderophore production [46,47]. Literature evidence indicates that *Enterococcus* can suppress pathogens by producing bacteriocins (e.g., enterocins), demonstrate antagonistic activity against fungi of the genera *Fusarium* and *Botrytis*, and act as strong competitors for essential nutrients such as iron and carbon [48]. *Enterococcus* species can enhance plant resistance to oxidative stress via antioxidant production and improve tolerance to salinity through the synthesis of osmoprotectants [49]. These bacteria can colonize seeds by entering through the root system from soil or via seed treatments, such as application of organic fertilizers [50]. However, some *Enterococcus* strains are opportunistic human pathogens (e.g., *E. faecalis*) and may carry antibiotic resistance genes [51].

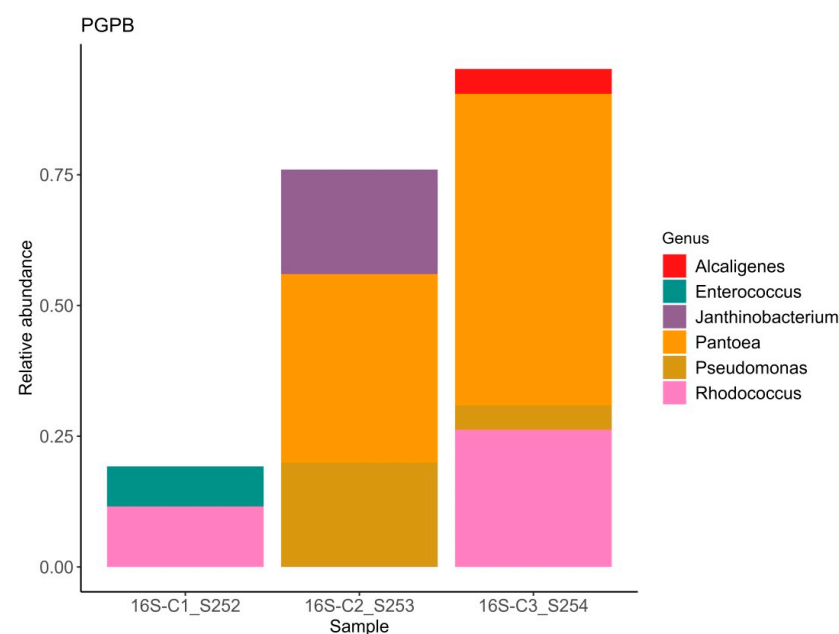
The genus *Alcaligenes* includes bacteria capable of colonizing plants and performing important ecological functions, such as plant growth promotion, atmospheric nitrogen fixation, phosphate solubilization, and synthesis of phytohormones (indole-3-acetic acid (IAA), gibberellins) [52,53]. *Alcaligenes* exhibits antagonistic activity against fungal pathogens (e.g., *Fusarium*, *Rhizoctonia*) and bacterial pathogens (e.g., *Pseudomonas syringae*) through the production of siderophores and antimicrobial compounds [54]. Additionally, *Alcaligenes* is capable of decomposing phenols and organochlorine compounds, as well as detoxifying heavy metals [55,56]. Consequently, *Alcaligenes* enhances plant resistance to salinity, drought, and heavy metal stress [49]. *Alcaligenes* is found in seeds, attributed to its ability to form biofilms and undergo vertical transmission from the parent plant [14].

It is well established that plants recruit beneficial microorganisms into the endosphere. Moreover, vertical transmission of the endospheric microbiome from the mother plant to the offspring via the seed microbiome has been documented in the literature [57,58]. This is why the study of seed endophytic microbial communities of plants, especially those consumed by humans in raw form, represents a promising direction in the development of biological plant protection agents. The number of studies reporting on the composition of seed endophytic microbial communities, including those of lettuce (*Lactuca sativa*), is increasing annually [59]. It has been shown that the endospheric bacterial community of lettuce seeds primarily consists of representatives from the phyla Pseudomonadota (formerly classified as Proteobacteria) and Bacillota (Firmicutes). The relative abundance of these major groups can vary considerably: Pseudomonadota accounts for approximately 40–89% and Bacillota for 10–60% of the community composition in non-germinated lettuce seeds [60]. At the genera level frequently detected *Pseudomonas*, *Pantoea*, *Bacillus*, *Serratia*, *Rahnella*, *Rhodococcus*, *Xanthomonas*, and *Streptomyces* [61]. Our data also demonstrate a predominance of bacteria from the genera *Pantoea*, *Rhodococcus*, and *Pseudomonas*, with substantial representation of bacteria from the genera *Candidatus Profftella* and *Janthinobacterium*. In this study the potential beneficial properties of bacterial genera comprising the endospheric microbial community were assessed. According to published data, nearly all representatives of the endospheric microbial community in lettuce seeds exhibit a range of beneficial traits, including plant growth promotion (PGPB), suppression of phytopathogens, enhanced stress tolerance, positive effects on seed germination, and detoxification of environmental pollutants. Interestingly, sample C1 exhibited the highest values of the Shannon and Simpson diversity indices (1.84 and 0.83, respectively) despite having the lowest relative abundance of beneficial microorganisms. In this sample, the relative abundance of bacteria with activity against phytopathogens was 19%, PGPB accounted for 19%, and bacteria enhancing stress resistance comprised 7% (Figures 1–3). Samples C2 and C3, which showed lower bacterial diversity (with Shannon and Simpson indices 1.2–1.1 and 1.7–1.5 times lower than those of sample C1, respectively), exhibited a substantially higher relative abundance of bacteria active against phytopathogens (56–95%), PGPB (76–95%), and stress-resistance-enhancing bacteria (64–76%). According to the literature, alpha diversity indices estimated for the endophytic bacterial community of lettuce seeds vary within the ranges of 0.3–1.0 for the Simpson index and 0.2–8 for the Shannon index [3,60,61]. It is important to note the limited scope of studies on the endophytic microbial community of lettuce seeds compared to those of crops such as rice and wheat [62–65].





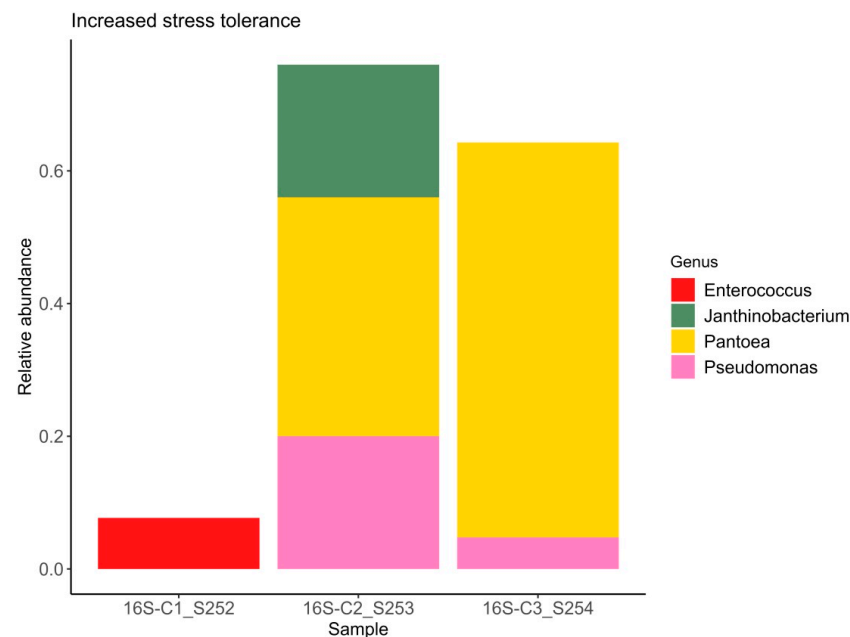
**Figure 1.** Relative abundance of bacterial ASVs with phytopathogen-suppressing capabilities.



**Figure 2.** Relative abundance of bacterial ASVs exhibiting plant growth-promoting traits.

Furthermore, it was demonstrated that seeds of the same variety, obtained from the same producer, may differ significantly in the composition of their endospheric bacterial communities. Overall, these results indicate that the lettuce seed endosphere harbors a microbiome of relatively low diversity, yet the constituent bacteria are capable of performing a broad spectrum of functions that support effective plant development. Specifically, the microbial community includes different types of PGPB: bacteria that synthesize phytohormones, siderophores; bacteria that fix nitrogen and solubilize phosphate; bacteria that suppress phytopathogens via three main mechanisms—production of antimicrobial compounds, competitive exclusion, and induction of systemic resistance; bacteria that enhance tolerance to abiotic stresses such as cold, drought, salinity, and oxidative stress; as well as bacteria that promote seed germination and detoxify pollutants including pes-

ticides, polycyclic aromatic hydrocarbons (PAHs), alkanes, heavy metals, phenols, and organochlorine compounds.



**Figure 3.** Relative abundance of bacterial ASVs capable of enhancing plant stress resistance.

## 5. Conclusions

The conducted research revealed the taxonomic groups and functional properties of the bacterial microbiome associated with lettuce seeds. It was established that endophytic bacteria constituting the seed microbiome possess significant potential to stimulate plant growth, enhance resistance to both biotic and abiotic stresses, and suppress phytopathogens. The endophytic bacterial community of lettuce seeds includes representatives of the genera *Pantoea*, *Rhodococcus*, *Candidatus Proffttella*, *Janthinobacterium*, *Pseudomonas*, *Enterococcus*, and *Alcaligenes*. Variation in the microbial community composition among samples indicates the influence of environmental factors on seed microbiome assembly. The results underscore the importance of further research into the functional characteristics of the seed microbiome and its targeted application in agricultural technologies aimed at increasing crop yield and product safety. The data obtained may serve as a foundation for the development of novel bioproducts based on beneficial endophytes that contribute to the sustainable advancement of crop production.

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**Data Availability Statement:** The original data presented in the study are openly available in sequence read archive (SRA) database under accession number PRJNA1279813.

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