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Core Bacterial Communities in the Endosphere of Lettuce (*Lactuca sativa*): A Comparative Study of Roots and Leaves

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Abstract. This study aimed to compare the endophytic microbiomes of lettuce leaves and roots grown under seven different environmental conditions, varying by substrate type (soil and hydroponics) and method of mineral nutrition. The core microbiome was defined as OTUs (Operational Taxonomic Units) present in all variants of leaves (roots) with a relative abundance > 0.3%. For lettuce leaves, only 4 common bacterial OTUs were identified, belonging to the genera *Lactobacillus* and *Sphingomonas*, and the family OPS 17. The core microbiome of lettuce roots included 9 OTUs: Bacteroidetes bacterium, OTUs from the family *Obscuribacterales*, and genera *Reyranella*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Aquabacterium*, *Pseudomonas*, env. OPS 17, and two different genera of *Sphingomonas*. *Notably*, *Sphingomonas* and env.OPS 17 were common to both leaf and root microbiomes. The results underscore the importance of considering both the endosphere and exosphere, as well as different plant parts, in microbiome studies. The distinct microbiomes of leaves and roots highlight the influence of environmental conditions and plant organs on microbial composition. These insights can inform strategies for optimizing plant health and growth through microbiome management, contributing to sustainable agriculture.

1 Introduction

Microorganisms inhabit both the surface and internal tissues of plants at every stage of their development, continuously interacting with the plant and influencing its resistance to environmental changes. For instance, it has been shown that microorganisms play a crucial role in promoting plant growth, health, and resilience by supplying nutrients, growth hormones, and other compounds that enhance development, suppress phytopathogens, induce systemic resistance, and expand the plant's tolerance to abiotic stresses [1-2]. The "plant-host" system and its associated microorganisms are so closely interconnected—whether in the phyllosphere (on the surface of above-ground parts), rhizosphere (in the root zone of the soil), or endosphere (inside the plant)—that they are viewed as a unified supra-organismal entity known as the holobiont [3-4]. Research on plant microbiomes has demonstrated that the composition of these microbial communities is influenced by factors

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such as plant genotype, cultivation substrate, climate, geographical conditions, and plant compartment [3, 5-6].

It has been observed that plants of the same species, regardless of their growing environment, consistently host a set of microorganisms that account for up to 75% of the species in the phytobiome [7]. These microorganisms, which are consistently associated with the plant, are referred to as the "core plant microbiome" [8]. The core microbiome refers to a stable community of microorganisms—such as bacteria, fungi, archaea, and viruses—that colonize a specific plant species or variety. This community is generally stable and plays a key role in the plant's health, growth, and development [9]. The core microbiome remains closely associated with the host, regardless of environmental conditions, and exhibits traits such as efficient colonization, nutrient absorption, and stress resistance [10]. It is widely believed that members of the core microbiome are vital to the host's biological functions and can significantly affect the health and nutritional value of agricultural crops [7].

Studies on the core microbiome of various agricultural plants have shown that in corn, bacterial taxa such as *Acidobacteria*, *Actinobacteria*, *Gammaproteobacteria*, *Alphaproteobacteria* and *Chloroflexi* account for 69.42%–85.89% of the core microbiome [8]. In rice, the core microbiome consists mainly of taxa from *Proteobacteria* and *Enterobacterales* [11]. In the wheat rhizosphere, the core microbiome (with a relative abundance greater than 0.5%) includes the genera *Roseiflexus*, *Flavobacterium*, *Gemmatimonas*, *Haliangium*, *Iamia*, *Flavisolibacter*, *Ohtaekwangia* and *Herpetosiphon* [12]. Despite significant progress in these crops, relatively little attention has been given to other plants, such as lettuce. However, lettuce is one of the most widely consumed raw vegetables. *Lactuca sativa* L. (lettuce) is one of the most frequently eaten fresh vegetables, providing an important source of fiber, vitamins, antioxidants, and other nutrients beneficial to human health. It is cultivated on approximately 1.8 million hectares under various conditions, including soil-based and hydroponic systems [13]. Additionally, lettuce is one of the most commonly used model plants in ecotoxicological, ecological, and vegetative studies.

Research on the core microbiome of plants is an emerging field, offering insights into how these microbial communities can be leveraged for agricultural benefits and ecosystem management. In this context, the present study aimed to evaluate the core endospheric bacterial microbiome of lettuce (*L. sativa*). The study involved plants grown on various substrates (soil and hydroponics) under different mineral nutrition conditions, encompassing seven different cultivation variants.

2 Materials and methods

2.1 Plant material and growing conditions

Lettuce seeds were used in this study. Vegetation experiments were conducted on two types of substrates – hydroponics (floating systems) and soil. Initially, the lettuce seeds were pre-germinated on vermiculite for 10 days to eliminate the influence of the environment on the microbiome of the seedlings. Afterward, seedlings were transplanted into opaque plastic containers (9 plants per container) either filled with hydroponic solution or soil. The vegetation period for all variants was 28 days. Environmental conditions were kept constant during the vegetation period: temperature at 22°C, light conditions (day: night) at 16:8 hours, and air humidity at 50-60%. The hydroponic solution contained the following macronutrients: $N{-}NO_3$ (14.0 mM), $N{-}NH_4^+$ (2.0 mM), P (2.0 mM), K (10.0 mM), Ca (4.5

mM), Mg (2.0 mM), S–SO_4 (5.0 mM) [14]. The hydroponic solutions were refreshed every 7 days.

For the soil-based vegetation experiment, soil was collected from a depth of 20-40 cm (coordinates: 55.790934, 49.120356). To ensure nutrient availability, mineral fertilizers were applied to the soil at the beginning of the vegetation period: superphosphate at 1.33 g/kg and potassium nitrate at 3.5 g/kg. The volume of the hydroponic solution and the mass of the soil in each container were 12 litters and 10 kg, respectively.

2.2 Fertilization and Sample Treatment

Micronutrients based on various chelating agents iminodisuccinate (IDS) and ethylenediaminetetraacetic acid (EDTA) were used for plant feeding. The micronutrients contained four metals in chelated form (IDS*Fe, IDS*Zn, IDS*Cu, IDS*Mn, EDTA*Fe, EDTA*Zn, EDTA*Cu, EDTA*Mn) and two in non-chelated form (H_3BO_3 and $(NH_4)_6Mo_7O_{24}$).

After the vegetation period, plants were removed from their growing substrates, washed, and parts of the above-ground and below-ground sections were sterilized and used for DNA extraction. Seeds, above-ground, and below-ground parts of lettuce plants were analysed separately. The variants of the analysed samples are presented in Table 1.

Table 1. Plant Samples Analysed in the Present Study.

Treatment Type	Growing Substrate			
	Hydroponics		Soil	
	Above-ground Part (Leaves)	Below-ground Part (Roots)	Above-ground Part (Leaves)	Below-ground Part (Roots)
Not fertilized (control)	H-A	H-B	S-A	S-B
Fertilized (IDS)	H-I-A	H-I-B	Sr-I-A	Sr-I-B
Fertilized (EDTA)	H-E-A	H-E-B	Sr-E-A	Sr-E-B
Fertilized (Mineral soil)	H-M-A	H-M-B	-	-

2.3 Statistical Analysis

All measurements were conducted with at least five replicates. Statistical analysis was performed using Microsoft Office Excel 2016 (Redmond, WA, USA).

3 Results

We conducted a comparison of the endophytic microbiomes of lettuce leaves and roots grown under seven different environmental conditions, varying by substrate type (soil and hydroponics) and method of mineral nutrition. The core microbiome included those OTUs (Operational Taxonomic Units) present in all variants of leaves (roots) with a relative abundance > 0.3%. For lettuce leaves, only 4 common bacterial OTUs were found (Table 2). These belong to the genera *Lactobacillus* and *Sphingomonas*, and the family OPS 17.

Table 2. Bacterial OTUs of the Core Microbiome of Lettuce leaf endosphere.

OTUs	H-A	H-I-A	H-E-A	H-M-A	S-A	S-I-A	S-E-A
f_env.OPS 17	1.69	2.11	2.16	3.47	2.07	1.49	1.41
g._*Lactobacillus*	1.11	26.34	24.39	10.40	0.62	0.40	1.03
g *Sphingomonas*	2.62	3.69	0.82	3.12	1.27	1.25	1.92
g. *Sphingomonas*	10.23	13.53	2.64	3.46	5.09	8.56	3.84

The core microbiome of lettuce roots included 9 OTUs: Bacteroidetes bacterium, OTUs from the family *Obscuribacterales*, genera *Reyranella*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Aquabacterium*, *Pseudomonas*, env.OPS 17, and two different genera of *Sphingomonas* (Table 3), with the last three also found in the core microbiome of leaves aptions should be typed in 9-point Times. They should be centred above the tables and flush left beneath the figures.

Table 3. Bacterial OTUs of the Core Microbiome of Lettuce root endosphere.

OTUs	H-B	H-I-B	H-E-B	H-M-B	S-B	S-I-B	S-E-B
s_Bacteroidetes bacterium	0.80	0.77	1.32	0.93	0.46	0.37	0.35
env.OPS 17	3.62	2.65	1.70	6.20	0.76	1.07	2.02
o_Obscuribacterales	0.55	1.45	0.40	0.66	0.34	0.44	1.52
f Reyranella	0.76	1.84	1.45	2.78	0.40	0.34	0.66
f Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	6.71	2.48	3.35	1.87	0.98	4.24	2.00
f Sphingomonas	1.34	2.10	1.47	2.14	1.44	1.30	1.71
f Sphingomonas	13.00	10.89	13.63	20.21	6.78	6.12	10.93
f Aquabacterium	0.60	1.42	1.40	4.47	2.07	2.08	3.57
f Pseudomonas	5.13	1.65	4.22	3.92	13.46	12.47	10.96

Additionally, when assessing the core microbiome, researchers use different components of the microbial associations with plants – some assess the totality of microorganisms in both endosphere and exosphere, evaluating roots and leaves together, while others focus only on one sphere or part of the plant [17]. This likely accounts for the differences in the composition of the core microbiome reported.

4 Discussion

The plant-host system and associated microorganisms are closely interconnected in the phyllosphere (on the surface of aboveground parts), rhizosphere (in the root zone of the soil), and endosphere (inside the plant). These interactions are considered within the concept of a supra-organismal entity known as the holobiont [1,18]. Improving yields, crop production quality, and other plant manipulations is only possible through the competent management of plant-associated microbial communities, their composition, and their functions. Next-generation sequencing methods have enabled a quantum leap in this area, but to date many questions remain open for a range of plant species. Over the past decades, the term core microbiome has become widely used in microbial ecology. The term core microbiome can be applied to a variety of microbial communities. In a broad sense, the core microbiome can be applied in general to the plant microbiome of a particular plant species [16-18].

Due to the close association of endophytes with plants, the use of endophytic microbiome as a biological control agent is a promising alternative to the use of chemical pesticides in the last few years. At the same time, the identification of representatives of the core microbiome allows us to determine the species with the highest functionality. In the present study, bacteria of the genus *Sphingomonas* had the highest abundance in the main endophytic microbiome of lettuce root and leaves. Bacteria of this genus are widely distributed in nature: they have been isolated from various terrestrial and aquatic habitats, from plant root systems, various specimens, and from the endosphere of plants. They are able to survive under conditions of low nutrient concentrations [19-20]. Thus, bacteria of this genus can be considered as biological control agents.

Generalizing the data of studies by other authors, it is possible to recommend agents for the creation of so-called synthetic microbial communities. For example, Damerum et al.

(2021) investigated the core microbiome (endosphere+exosphere) of lettuce leaves. They identified 45 ASVs belonging to five phyla: *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Chloroflexi* and *Deinococcus-Thermus*, with the relative abundance of 32 out of 45 being <1%. The thirteen most abundant ASVs (≥1%) belonged to the genera *Pseudomonas*, *Buchnera*, *Alkanindiges*, *Massilia*, *Acinetobacter*, *Sphingomonas*, *Duganella*, *Methylobacterium*, *Pantoea*, *Xanthomonas*, *Aquabacterium*, *Methylophillus*, *Deinococcus*, *Chryseobacterium* and *Aquitalea* [21]. It is just as important to understand the core endophytic microbiome of other plants. Other studies have noted that the core endophytic microbiome of *Arabidopsis* roots is dominated by *Actinobacteria*, followed by *Proteobacteria*, *Bacteroidetes* and *Firmicutes* [3, 22]. Similar results were obtained when analysing endophyte communities in the roots of tomato and sugarcane [23.24]. It is important to note that *Streptomycetales* and *Pseudomonadales* were highly enriched and predominant endophytes in tomato roots, while *Micromonosporales*, *Rhizobiales*, *Sphingomonadales*, *Burkholderiales*, *Xanthomonadales* and *Flavobacteriales* were also among the most prevalent bacterial groups [23]. Further studies on the interactions between plants and their major members of the microbiome will contribute to further practical applications in agriculture.

5 Conclusion

This study highlights the complexity and variability of the core microbiomes in lettuce leaves and roots under different environmental conditions. The findings reveal distinct core microbiomes for leaves and roots, with a few overlapping taxa such as *Sphingomonas* and env. OPS 17. For lettuce leaves, the core microbiome included only 4 bacterial OTUs, while the roots harboured a more diverse core microbiome comprising 9 OTUs. This variability underscores the importance of considering both the endosphere and exosphere, as well as different plant parts, when assessing core microbiomes.

The observed differences in microbial composition suggest that both environmental conditions and the specific plant organ being studied significantly influence the structure of the microbiome. This has important implications for agricultural practices and the development of strategies aimed at optimizing plant health and growth through microbiome management.

Additionally, the comparison with other studies on different plants like *Arabidopsis*, tomato, and sugarcane demonstrates common patterns in endophytic microbiome composition across different species, though specific dominant taxa can vary. These insights contribute to a broader understanding of plant-microbe interactions and the role of the microbiome in plant health and development.

Future research should continue to explore the functional roles of these core microbiome members and their potential applications in sustainable agriculture. Understanding how different environmental factors shape the microbiome can help in developing targeted approaches to enhance crop resilience and productivity.

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