Microorganisms in aquaponics: insights on the composition of the root microbiome of lettuces of varying age

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Abstract

Aquaponics is a developing, soilless production technique combining hydroponics and recirculating aquaculture and is now spreading worldwide. Nevertheless, several aspects of aquaponics still need research. Indeed, despite being key-players in the dynamic equilibrium of aquaponic systems, microorganisms and their roles in aquaponics are still scarcely known. The aim of this study is thus to explore the microorganisms communities thriving in the root compartments of lettuce in the closed-loop aquaponic system of Gembloux Agro-Bio Tech and to focus on the differences between the microbial communities of lettuce of varying age. Therefore, root samples were collected from lettuces of five different age groups and microorganisms from the rhizoplane and from the endosphere were harvested. DNA was then extracted and sequenced on an Illumina MiSeq platform, targeting the V1-V3 region of the 16S rRNA gene. Results show that no significant difference could be noted between the different age groups despite a visible trend on the Bray-Curtis PCoA. However, significant differences in alpha- and beta-diversity could be observed between the rhizoplane and endosphere compartments. In terms of taxonomy, the composition of the root community is similar to what can be found in the literature and coherent with the previous experiments conducted in the same aquaponic system.

Keywords: aquaponics, bacterial communities, rhizoplane, endosphere, lettuce roots, diversity, 16S rRNA, NGS

INTRODUCTION

Aquaponics is an innovative production technique spreading worldwide which combines recirculating aquaculture and hydroponics (Junge et al., 2017). Albeit soilless, a parallel can be drawn between aquaponics and soil when it comes to the importance of microorganisms. Indeed, just as in natural ecosystems, microorganisms are known to be key players in aquaponics, involved in processes such as nitrification but also plant health and care (Eck et al., 2019; Sanchez et al., 2019). Thanks to recent advances in sequencing technologies, the study and exploration of microbiomes became more accessible and spread to a wide range of scientific fields (Munguia-Fragozo et al., 2015). In aquaponics, several studies have already dealt with the microbiome (Schmautz et al., 2017; Bartelme et al., 2019; Eck et al., 2019; Sanchez et al., 2019) and started exploring the composition of the aquaponic microbial communities. Indeed, Schmautz et al. (2017) first analysed the taxonomic composition of several compartments of their aquaponic system thus giving a first insight into the composition of the aquaponic bacterial communities, while Eck et al. (2019) carried out a complementary approach in studying the bacterial communities of the sump and biofilter compartments of varying aquaponic systems in Europe. Meanwhile, Sanchez et al. (2019) tackled a different angle of the microorganisms study in aquaponics by focusing on the potentially plant beneficial functions harbored by the bacterial communities in aquaponics. On the other side, Stouvenakers et al. (2020) investigated the suppressiveness activity of aquaponic water on specific plant pathogens such as *Pythium aphanidermatum*. Furthermore,

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it is now known that the plants present in the system can greatly influence the composition of the microorganisms communities as shown by Eck et al. (2019) when comparing the same system with and without the hydroponic compartment and as confirmed by Bartelme et al. (2019). In this study, we chose to focus on the root microbiome of lettuces grown in an aquaponic system and more particularly on the bacteria present on the roots of lettuces of varying age.

In their experiment which dealt with *Arabidopsis thaliana*'s roots microbiome in soil, Chaparro et al. (2014) compared the root microbiome of *Arabidopsis* at contrasted physiological stages (seedling, vegetative, bolting and flowering). Significant differences were observed for the *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Cyanobacteria* phyla between the several physiological stages but no major differences were observed for the other phyla. Trends were also observed by Sugiyama et al. (2014) when comparing rhizosphere communities in soybean fields at vegetative, flowering and mature stages and the bacterial communities of the three stages seemed to be composed of different microorganisms.

The main hypothesis explaining these changes is that depending on the physiological stage, the plant secretes different exudates aimed at attracting specific microorganisms involved in stage specific functions (Chaparro et al., 2014; Sugiyama et al., 2014). This hypothesis has been studied in a great variety of plants such as medicago, maize, pea, wheat and sugar beet (Baudoin et al., 2002; Mougel et al., 2006; Houlden et al., 2008; Micallef et al., 2009).

The question now remaining is whether such a phenomenon can be observed for lettuces in soilless systems as well, and more precisely in aquaponics.

MATERIAL AND METHODS

This comparison of lettuce roots' microbiome between plants of varying age is a part of a wider experiment which aimed at following the evolution of the microbial communities present in an aquaponic system over the course of a full lettuce growth cycle.

Aquaponic system

The aquaponic system used in this experiment (Figure 1) has already been described in details in Eck et al. (2019, 2020). Briefly, it is a coupled system composed of two 400-L fish tanks in which tilapias (*Oreochromis niloticus*) were reared. The water flows from these fish tanks, through a lamellar settler and a pressurized microbeads biofilter before being pumped up to the plants. The hydroponic compartment is composed of four floating rafts through which the water flows before falling back to the fish.

The experiment took place in 2019. Lettuces were sown on February 29, March 7, March 14, March 21 and March 28 in a controlled environment greenhouse. Seeds of *Lactuca sativa* 'Lucrecia' (Rijk Zwaan) were placed in rockwool plugs (Grodan Rockwool B.V., Roermond, the Netherlands) which were then deposited in shallow boxes filled with tap water. Eleven days after sowing, the obtained seedlings were transplanted into the aquaponic system.

Figure 1. Plant and fish farming box, Gembloux Agro Bio-Tech. From the outside it is possible to see the maritime container topped up by the greenhouse (a). From the inside, one can observe the two fish tanks (b) and the raft hydroponics growing leafy greens (c). Source: Eck et al. (2020).

Root sampling and microorganisms collection

On April 29, roots were collected on lettuces from varying age: 3 weeks old, 4 weeks old, 5 weeks old, 6 weeks old and 7 weeks old. 0.2 g of roots per sample were collected in the 3-weeks and 4-weeks old groups due to a lack of tissue while 2 g of roots per sample were collected in the 5-weeks, 6-weeks and 7-weeks old groups. Each root sample was then washed in KPBT buffer (0.05 M potassium phosphate, 0.005% Tween 80, pH 6.5) for the collection of the rhizoplane community according to the protocol developed by Sare et al. (2020). 30% of glycerol was added to each rhizoplane community sample which were flash frozen and stored at -80°C until DNA extraction. Meanwhile, the washed roots were also flash frozen and stored at -80°C until further steps for endophytes harvest. For the collection of the endosphere community, roots were defrosted in a heat chamber at 55°C for 5 min and placed in a mesh bag (Linaris Biologische Produkte GmbH, Dosenheim, Germany) with a ratio of 9 mL of KPBT buffer g^{-1} of root. The roots were then grinded and homogenized in the mesh bash using a tissue grinder. The mashed solution was collected and filtered through a sterile cheesecloth in a 50-mL Falcon tube, in order to get rid of leftover root pieces. 30% of glycerol was added to the solution in order to be stored at -20°C until DNA extraction.

DNA extraction and sequencing

The DNA extractions were conducted following the method of Eck et al. (2019) using the Fast DNA Spin Kit (MP Biomedicals) and the Cell Lysis TC solution. Before being stored at -20°C, the DNA concentration from each sample was estimated using a Nanodrop (NanoDrop ND-1000 Spectrophotometer, NanoDrop Technologies, Wilmington, DE, USA) which also provided the 260/280 and 260/230 ratios of absorbance. These data were used for the quality control required for the sequencing process. After quality control, amplicons libraries were prepared. Each DNA sample was first diluted ten times in ultra-pure water. Then, a PCR amplification of the V1-V3 hypervariable region of the 16S rRNA gene was performed using the following primers: 27F 5' **TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGAGTTTGATCC TGGCTCAG-3'and 534R 5'-**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ATTACCGCGGCTG CTGG-3' with the Illumina sequencing adapters in bold. PCRs were performed using the 2X KAPA HiFi HotStart ReadyMixPCR kit (KAPA Biosystem). Reactions of 25 µL were used, containing 5 µL of primer forward and 5 µL of primer reverse both concentrated at 1 µM, 12.5 µL of 2X KAPA HiFi HotStart ReadyMix, a high-fidelity enzyme and 2.5 µL of DNA sample. PCRs were performed with the following cycling protocol: a pre-heating process of the lid at 110°C; a denaturation step at 95°C for 3 min; 25 cycles of 30 s at 95°C; an annealing step of 30 s at 55°C; an extension step of 30 s at 72°C; a final extension step of 5 min at 72°C. The samples were then kept at 4°C until proceeding further.

Sequencing was then performed by the DNA Vision company (Gosselies, Belgium) on a Miseq (Illumina) with paired-end 2×250 bp length.

Bioinformatics

Bioinformatics analyses were performed with the QIIME 2 2019-4 pipeline (Bolyen et al., 2019) on the forward reads only, following the workflow and parameters detailed in Sare et al. (2020). Briefly, quality filtering and trimming were performed with DADA2 while taxonomy assignment was carried out via VSEARCH and the Silva_132 database. The features table was rarefied at 3744 reads and the diversity analyses were performed at the same cutoff.

RESULTS AND DISCUSSION

Comparison of the two root compartments and the varying lettuce age

As shown in Figure 2, rhizoplane and endosphere samples clustered in two distinct groups. According to a permanova pseudo-F test, the rhizoplane and endosphere compartments are significantly different from each other (q-value=0.009). This difference was confirmed by the same permanova pseudo-F test conducted on the weighted unifrac distance matrix (q-value=0.037). Furthermore, a Kruskal-Wallis test confirmed that the Shannon index and the number of observed otus (richness) were significantly different in both

compartments, indicating a higher diversity in the rhizoplane than in the endosphere. The same diversity gap was observed by Dong et al. (2019) and Poudel et al. (2019).

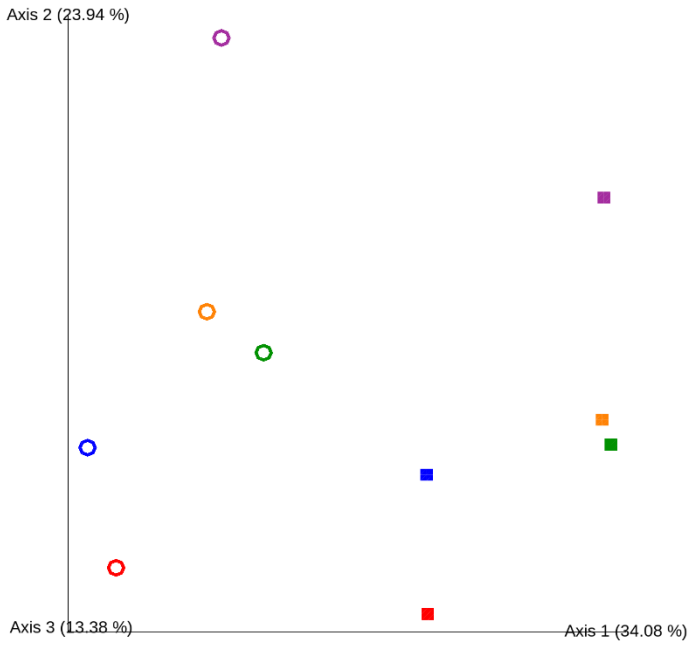

Figure 2. Principal coordinates analysis (PCoA) plots of the Bray-Curtis distance matrix. Circles = rhizoplane, squares = endosphere. Red = week 3, blue = week 4, orange = week 5, green = week 6 and purple = week 7.

Concerning the age groups, no statistical test could be conducted as only one sample of each age per compartment was taken. However, trends can be observed with the axis 2 clearly separating the samples by age. This trend can moreover be observed in both compartments. According to a Spearman correlation test conducted on the Shannon index and the number of observed otus, the alpha diversity does however not change with the age group.

Composition of the samples

Figure 3 provides a first insight into the composition of the bacterial communities present in the lettuce roots compartments at varying age. An ANCOM test was performed both at the phylum and genus levels to identify the taxa responsible for the differences between compartments noted on the PCoA (Figure 2). At the phylum level, the *Actinobacteria*, *Deinococcus-Thermus*, FCPU426, WPS-2 and *Cyanobacteria* phyla are significantly different between both compartments. At the genus level, no major taxa were found to be significantly different between both compartments. Trends can however be distinguished. Indeed, the *Hydrogenophaga* genus is much more present in the endosphere with an average of 11% of the reads (±3.6%) compared to the rhizoplane were it represents only 2.6% of the reads (±0.9%). This genus was previously part of the *Pseudomonas* taxa (Willems et al., 1989) and harbors hydrogen-oxidizing bacteria. The *Flavobacterium* genus is slightly more present in the endosphere than in the rhizoplane (with an average of 10.5±5.8% versus 7.6±6.0%) but its proportion seems highly dependent on the age of the lettuce. Conversely, the *Burkholderiaceae* family is slightly more present in the rhizoplane than in the endosphere (6.1±1.8% versus 4.2±1%, respectively).

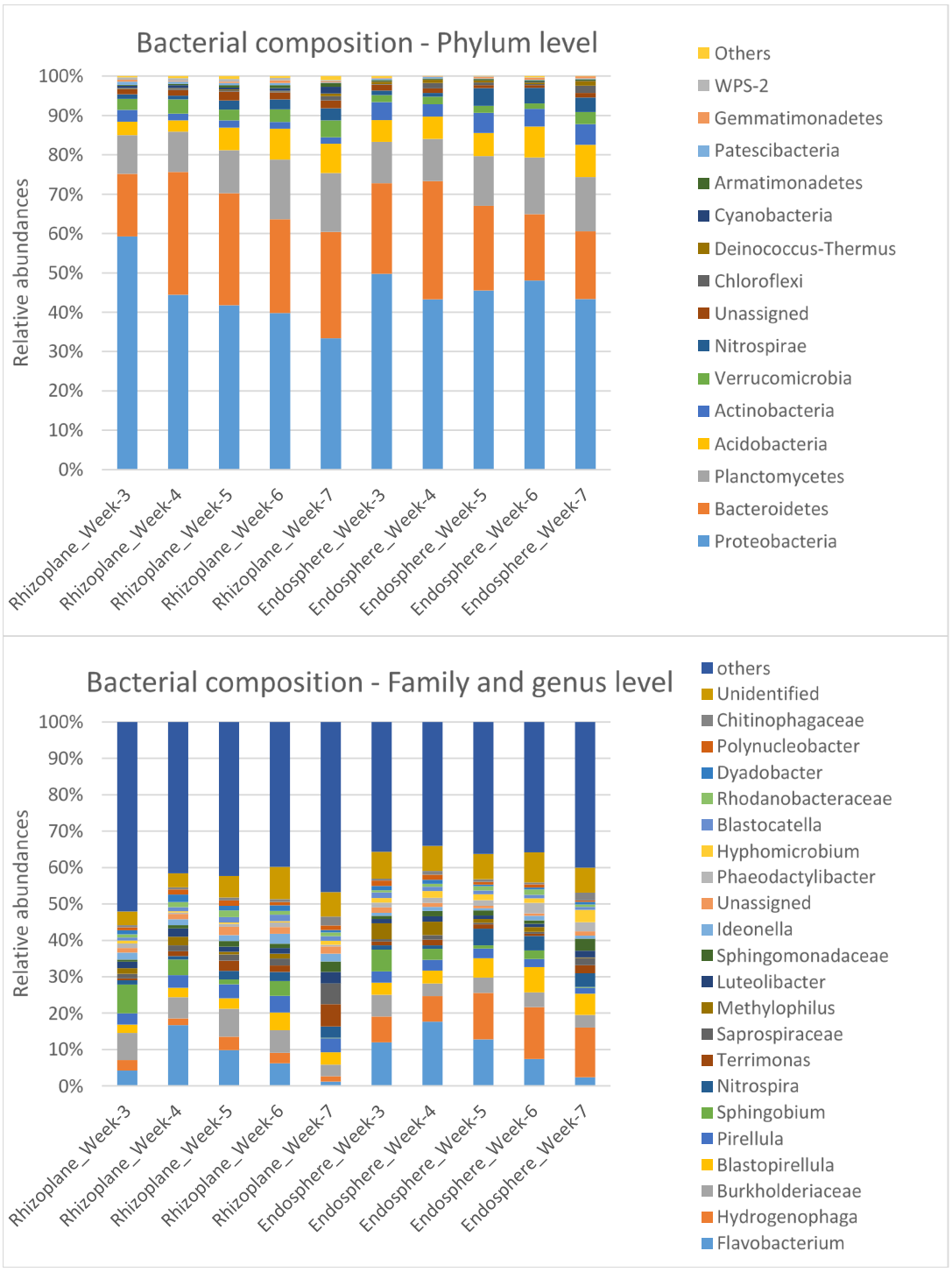

Figure 3. Overview of the taxonomic profile at the phylum and family and genus levels of the rhizoplane and endosphere compartments. At the phylum level, only the phyla representing more than 0.2% of the total reads are presented while the rest is grouped in 'others'. At the family and genus level, the selected cutoff is 1%.

Overall, the global composition of the root compartment in aquaponics seems similar to what can be found in soil in other plants (Chaparro et al., 2014) but also in lettuce (Cardinale et al., 2015). Samples are dominated by the *Proteobacteria* phylum. An important proportion of *Bacteroidetes* can also be noted. *Bacteroidetes* were found to be highly present in aquaponic

systems in the water and biofilter compartments (Eck et al., 2019).

At the genus level, the composition of both compartments is also coherent with previous results obtained from the same aquaponic system (Eck et al., 2019, 2020; Stouvenakers et al., 2020). Indeed, the predominant families/genera are the *Burkholderiaceae* family, the *Flavobacterium* genus and the *Hydrogenophaga* genus, albeit in different proportions in both compartments. This stability is an interesting information as it shows that despite different experiments being run in different seasons in this system, the root communities are stable within the aquaponic system and lettuce root system.

Between the different age groups a few trends can still be noted. The *Burkholderiaceae* family proportion seemed quite similar between the different age groups, while *Flavobacterium* fluctuated a lot. Its peak was found in the 4-weeks-old group (16.72% of the four-week-old sample in the rhizoplane and 17.71% in the endosphere) and its proportion then gradually decreased with the plant age, attaining 1.2% in the 7 weeks old group in the rhizoplane and 2.4% in the endosphere. In the experiment of Qin et al. (2016) on wheat, *Flavobacterium* strong presence was correlated to early growth stages. Here, *Flavobacterium* proportion did gradually decrease with later growth stages but the first 3-week-old group did not have the most abundant proportion.

CONCLUSIONS

To conclude, this study confirmed that the rhizoplane and endosphere bacterial communities of lettuce are indeed different from one another in aquaponics. However, no significant changes in the composition of the bacterial communities could be distinguished between the different age groups despite a trend being observed on the PCoA based on the Bray-Curtis distance matrix. A comparison of the root microbial communities should be conducted again on more distinct groups of age, i.e., at contrasted physiological stages. Furthermore, repeating this experiment with different plant species would also enable to assess the capacity of aquaponic water to standardize the root microbiome over time.

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