Metagenomic Analyses of Rhizosphere Bacteria from Peace lily Exposed to Atmospheric Benzene.

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Introduction

Volatile organic compounds (VOCs) in indoor air are known to elicit various minor and major health impacts in man. Many plant species have the capacity to eliminate VOCs from indoor air *via* a process called phytoremediation. Microorganisms in the root zone (rhizosphere) of plants are considered the principal site of VOC degradation and can account for up to two-thirds of the total VOC removed from the environment (Orwell *et al.*, 2004). Plants have been ranked as superior, intermediate or poor eliminators of VOC (Yang *et al.*, 2009). Such functional variation between plants is thought to be due to differences in the microbiome of their rhizosphere.

In this study, the common indoor plant species ***Spathiphyllum wallisii*** (Peace lily) was exposed to benzene in static test chambers. The rhizosphere microbiome was characterised through 16S rRNA sequencing workflow (Illumina MiSeq) to determine species abundance. Comparison of species abundance has been made between plants which were not exposed to benzene and to the growth medium (John Innes number 2) in which no plants have been grown. These early observations are part of a wider study to enhance our understanding of the role of the rhizosphere microbiome in VOC detoxification and may assist in the optimisation of phytoremediation systems for specific indoor environments.

Method

Potted Peace lilies (N=4) were maintained in static test chambers formed from modified glass fish tanks. Two plants were exposed to 10 ppm benzene (C_6H_6), which was injected into the chamber daily for four weeks. A further two plants were maintained under the same environmental conditions without benzene administration as experimental controls.

At the end of the four week treatment period, rhizosphere DNA was extracted from all plants using the PowerSoil DNA extraction kit (Cambio). Bacterial 16S variable regions 3 and 4 were amplified (Probio Uni and Probio Rev primers) by PCR and the resulting amplicons sequenced (Illumina MiSeq). Bacterial identification, to species level, was performed using USEARCH Version 6 (Edgar, 2010).

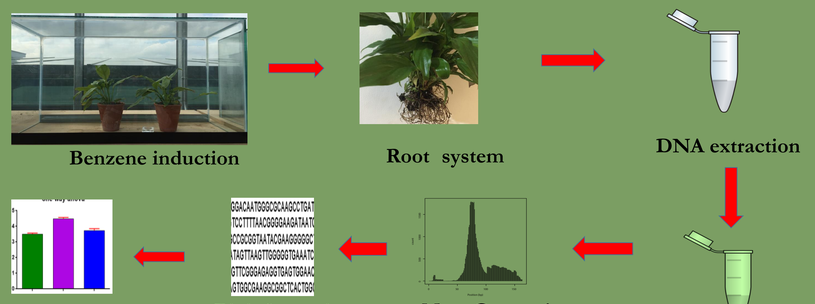
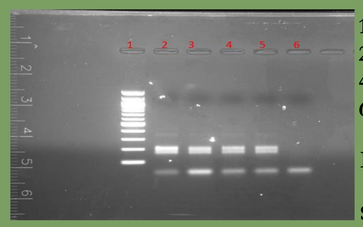


Figure 1: Workflow involving in the method

Results

Figure 2 shows presence of 16S rRNA gene in soil DNA samples. The expected size of the bands was 194bp according to the probio primer's amplicon size.



1-100bp ladder
2, 3-DNA from *S.wallisii*, C_6H_6 treated
4, 5-DNA from *S.wallisii*, without C_6H_6
6- PCR control (No DNA)

Figure 2: Agarose gel image shows presence of bacterial 16S rRNA gene in rhizosphere from Soil samples

Figure 3 shows percentages of total sequence reads in the top 5 OTU of DNA sequences. The difference between the John Innes and rhizosphere soil DNA sequences indicates the presence of Peace lily has an influence on the soil microflora. All rhizosphere DNA samples show the presence of ***Sphingobium*** and ***Halospirulina*** species (except 4). OTUs of John Innes number 2 soil DNA represent mixed species which have very similar identities.

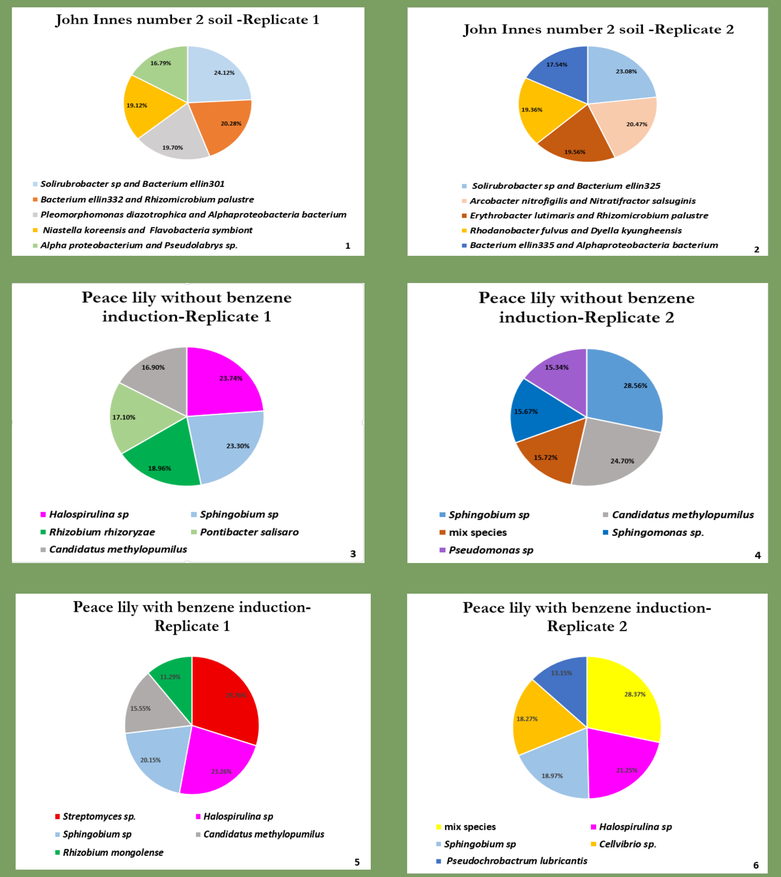


Figure 3: OTU distribution: Number of sequencing reads in each OTU represent species of bacteria, expressed as the percentage of the top 5 OTUs.

Figure 4: Indoor Green wall system, Staffordshire University

Discussion

Illumina MiSeq provides a comprehensive view of the soil bacterial population which cannot be obtained by performing culture dependent analysis.

Analysis of percentage of the first 5 OTUs of John Innes soil DNA sequences and Peace lily rhizosphere DNA sequences, clearly show the presence of plants has had an effect on the composition of the soil bacteria.

Though it was expected to see the difference of bacterial composition between benzene treated and untreated plants, our results do not show major differences between the dominant species in those samples. The main reason for this could be that the benzene concentration was very low and the benzene-plant exposure time was not enough to see the specific bacterial community changes. Therefore in the next experiment, higher concentration of benzene (100ppm) will be induced into the Peace Lily and sequence bacterial metagenomic DNA of rhizosphere to distinguish between benzene treated and untreated plants.

Work in Progress

- Benzene (100 ppm) induction - Peace lily experiment
- 10 ppm and 100 ppm benzene induction -Spider Plant and English ivy
- Determination of VOC degradation efficacy of each plant species
- Determination rhizosphere changes in each species

References

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