Isolation and Characterization of Volatile Organic Compounds Metabolizing Bacteria from Rhizospheres of Indoor Plants

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---------- INTRODUCTION ----------

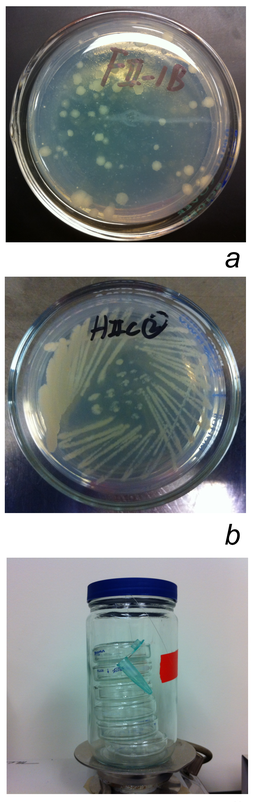
- Volatile Organic Compounds (VOCs) are of concern for indoor air quality due to their potential to cause respiratory related ailments.
- Studies have found that a large variety of plants used as indoor ornamental plants have the capacity to remove the VOCs from indoor air [1]. It has also been documented that the microbial community in the rhizosphere contributes to the VOCs removal, but this role has not been well investigated.
- The purpose of this study is to isolate and characterize bacteria from the rhizospheres of commonly used indoor foliage plants for enhanced reintroduction later.

a *b* *c* *d*

Figure 1. Indoor foliage plants used in the study: *Fittonia argyroneura (a&c) and Hoya carnosa (b&d).*

---------- METHODS AND MATERIALS ----------

- **Plant Preparation**
  Two indoor ornamental plants (Figure 1) were exposed to toluene for 8 hrs a day for 2 months in an enclosed container to encourage the growth of VOCs degrading bacteria.
- **Isolation of the bacteria**
  Rhizosphere samples (Figure 1*c&d*) were suspended in phosphate buffer and used to prepare serial dilutions. The serial dilutions were plated on mineral salt medium (MSM) agar and incubated in air tight mason jars (Figure 2*a-c*) with toluene as sole carbon source. The dilutions were also cultured in liquid MSM. Well isolated single colonies were picked and re-streaked multiple times to achieve pure cultures (Figure 2*a&b*).
- **Genetic screening of the bacteria**
  BOX-PCR was used to genetically screen the isolates for further characterization [2]. Banding pattern analysis (Figure 3) of the isolates' genetic fingerprints was done with the *GelCompar II* software. Eighty % similarity in banding pattern was used as a cut-off.
- **Identification of bacteria**
  Isolates with unique fingerprints were identified based on their partial 16s rDNA sequences, which were processed with the *Clustal Omega* software.



a *b* *c*

Figure 2. Bacterial cultures from rhizosphere of *Fittonia argyroneura (a&b)*, incubation set-up (c)

---------- RESULTS ----------

- Forty two isolates were acquired from both plants species, 12 of which were from *Hoya carnosa*, and 30 were from *Fittonia argyroneura*.
- BOX-PCR banding pattern analysis revealed (Figure 3) 23 unique isolates with less than 80% similarity for further characterization.
- Analysis of 16s rDNA sequence data showed the isolates had the highest % similarity with 8 known bacterial strains (Table 1).
- The phylogenetic relationship among the isolates is shown in Figure 4.

Table 1: Isolate counts from the rhizospheres of the indoor plants and the corresponding bacterial strains with which they had the highest percent sequence similarity.

No. of isolates from *Hoya carnosa*	No. of isolates from *Fittonia argyroneura*	Bacterial strain with which the isolates had the maximum sequence similarity
2	0	*Paenibacillus lautus* strain JCM 9073
1	1	*Paenibacillus tundrae* strains Ab10b
1	11	*Microbacterium aerolatum* strain V-73
0	1	*Rhodococcus marinonascens* strain DSM 43752
0	2	*Paenibacillus barcinonensis* strain BP-23
0	2	*Paenibacillus taichungensis* strain BCRC 17757
0	1	*Microbacterium kribbense* strain MSL 04
0	1	*Paenibacillus favisporus* strain GMP01

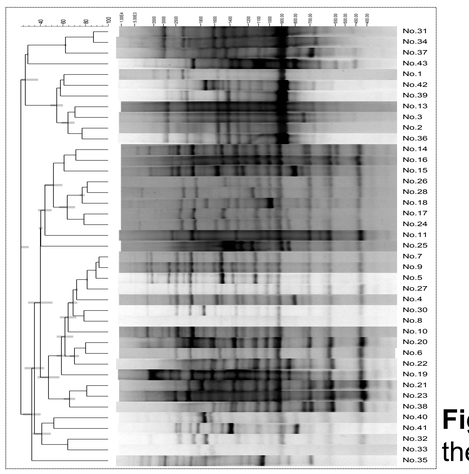


Figure 3. BOX-PCR Fingerprints of all the isolates.

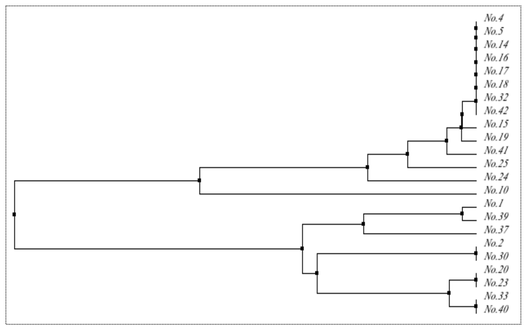


Figure 4. Phylogenetic tree of the bacterial isolates based on partial 16s rDNA sequence.

---------- CONCLUSION and ONGOING WORK----------

- The isolation and characterization of bacterial strains that were capable of degrading toluene confirms previous speculations that the rhizosphere microbial community contributes to the phytoremediation potential of indoor foliage plants.
- Using ^{14}C-labeled toluene, we are characterizing the growth kinetics of the isolates (i.e., toluene use efficiency). Isolates will be reintroduced into the rhizosphere based on their growth parameters to enhance the plants' phytoremediation potential.

---------- REFERENCES ----------

1. Orwell, R.L., R.L. Wood, J. Tarran, F. Torpy, and M.D. Burchett. 2004. Removal of benzene by the indoor plant/substrate microcosm and implications for air quality. Water Air Soil Pollution 157: 193-207.
2. Bart J.G., Daniel, G., Mark, M., Linda, W. Using BOX-PCR to exclude a clonal outbreak of melioidosis. 2007. BMC Infectious Diseases. 7: 68.