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Introduction

- Phytoremediation is the use of plants to remediate contaminated soils. Ideally, plants should have deep roots, be perennial, and use large quantities of water. Poplar and Willow trees are the most commonly used. Some pollutants, however, are not metabolised by plants and, thus, accumulate and cause phytotoxicity. Phytoremediation, alone, may not be a suitable technology for all contaminated soils.
- Bacterial soil bioremediation can be reduced significantly in time by the ability of the indigenous bacterial population to overcome any introduced species.
- “Endegrade” is an EU funded project which is attempting to utilise endophytes (bacteria living within plants) to breakdown pollutants as they are translocated through the plant, thereby reducing phytotoxicity and volatilisation (Patent :van der Lelie *et al*, 2001).

Table 1. List of pollutants not metabolised sufficiently in planta.

Compound	Fate in plant (toxic, build up or volatile)	Reference
Phenols	Toxic	Pfleeger <i>et al</i> , 1991
Chloro-phenols	Toxic	Pfleeger <i>et al</i> , 1991
TNT	Toxic, degraded to amino-dinitrotoluene	Thomson <i>et al</i> , 1998
Amino-dinitrotoluene	Rather persistent, toxic	Thomson <i>et al</i> , 1998
MTBE	Volatile	Trapp <i>et al</i> , 1994
BTEX	Volatile	Trapp <i>et al</i> , 1994
TCE	Volatile, build up of trichloroacetate	Trapp <i>et al</i> , 1994
PER	Volatile	Trapp <i>et al</i> , 1994

Aims :

- Little is known about the bacteria that live within plants, and until recently it was thought that all were pathogenic (Lodewyckx *et al*, 2002). In order to successfully identify and re-introduce bacteria that can survive in plants, we need to understand the community dynamics of endophytes.
- This study aimed to isolate bacteria from within the xylem sap of Willow and Poplar trees, culture and identify them by 16S rDNA sequencing and ascertain the community structure of the culturable microbial population.
- Dominant, culturable bacteria are being screened to avoid pathogens, identify species that can degrade problem pollutants, or to be used to introduce degradation genes, such as the *pTOM* plasmid.

Hypothesis:

- Different tree species will have different diversity of bacterial endophytes, and these will be specific to the plant compartment sampled.
- Endophytic bacteria will be able to naturally degrade pollutants as they pass through the plant or can be engineered to enhance their ability to do so.

Figure 1. Installation of a phytoremediation barrier for the treatment of a BTEX-TCE plume. Phytoremediation will be done using hybrid poplar. Phytoremediation will be improved using modified endophytic bacteria.



Figure 2- Endophytic bacteria isolated from Poplar and Willow trees at Figure 1 phytoremediation site, classified by 16S rDNA sequencing to genus level.

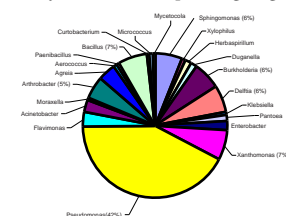


Figure 3- Classification of the Pseudomonas species found within the endophytic bacteria isolated from Poplar and Willow.

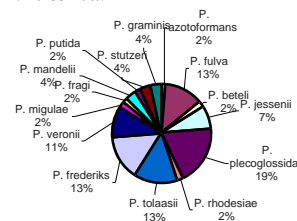


Figure 4 - Number of isolates from Willow, in relation to the location within tree.

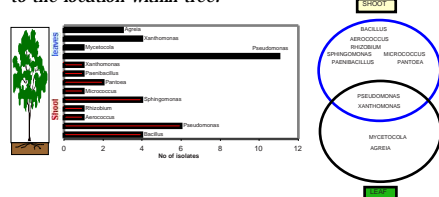


Figure 5 - Number of isolates from Poplar var. Hazendans, in relation to the location within tree.

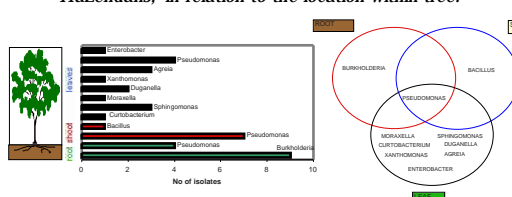
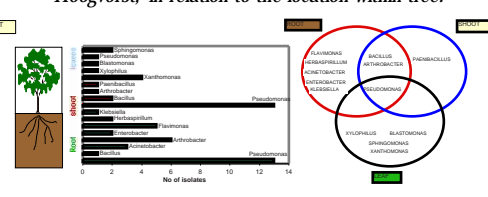


Figure 6 - Number of isolates from Poplar var. Hoogvorst, in relation to the location within tree.



Discussion:

A wide range of Eubacteria were found in all tree types, including a few pathogenic strains, with more diversity than was expected. The majority of the isolates were *Pseudomonas* species, which are known degraders of many potential target pollutants (Table 1). Importantly, this genus was present throughout all compartments of the plants studied. There were however distinct differences in the diversity of endophytes between different plant tissues (Fig 4-6).

Conclusions & future work:

- A similar study on the non-culturable endophytic community is on-going to establish whether the information gained from studies on isolates is representative of the total bacterial population within the plant.
 - A number of isolates have been found to have the natural ability to degrade toluene, and to have a natural resistance to a range of antibiotics. *Burkholderia cepacia* has also had the *pTOM* plasmid introduced successfully, which encodes for toluene degradation, and re-inoculation studies are continuing. At present, studies are ongoing to introduce GFP, *rfp*, *lux* and heavy metal resistance markers into strains possessing natural degradation abilities.
 - We are currently assessing the seasonal changes in the endophytic communities of Willow and Poplar, at the inter- and intra-species levels. As Figures 4-6 above shows, a considerable amount of compartmentalisation occurs within the plants, with different bacterial species being found within the same compartment of the different tree variety or species.
- Endegrade provides a real possibility to enable phytoremediation to tackle a greater range of polluted environments, while enhancing its social

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Patent registered:
D.Van der Lelie, S.D’Haene, D.N. Dowling, U. Karlson, E.R.B. Moore, S. Taghavi, S.A.J. Trapp, J. Vangronsveld. “METHOD FOR IMPROVING PHYTOREMEDIATION TREATMENT OF A CONTAMINATED MEDIUM”. Application nr. 60/291.344. U.S. Patent office. 16 May, 2001.

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