Identification of Plant Derived Substances That Enhance Biodegradation of Polycyclic Aromatic Hydrocarbons In Soils

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Phytoremediation Phytoextraction

Rhizofiltration Phytostabilization

Rhizoremediation Phytovolatilization

Phytobial Remediation

Plant Common Name (*Genus, Species*) [Family]	**Petroleum Hydrocarbons**	**Mechanism of Phytoremediation**
Western wheatgrass (*Agropyron smithii*)	chrysene, benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a,h*]anthracene	unknown
Big bluestem (*Andropogon gerardi*) [Gramineae]	chrysene, benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a,h*]anthracene	unknown
Side oats grama (*Bouteloua curtipendula*)	chrysene, benzo[*a*]pyrene benz[*a*]anthracene, dibenz[*a,h*]anthracene	unknown
Blue grama (*Bouteloua gracilis*)	chrysene, benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a,h*]anthracene	unknown
Common buffalograss (*Buchloe dactyloides*)	naphthalene, fluorene, phenanthrene	unknown
Prairie buffalograss (*Buchloe dactyloides)*	naphthalene, fluorene, phenanthrene	unknown
Canada wild rye (*Elymus canadensis*)	chrysene, benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a,h*]anthracene	unknown
Red fescue (*Festuca rubra* var. Arctared)	crude oil and diesel	rhizosphere effect
Poplar trees (*Populus deltoides* x *nigra*)	potential to phytoremediate benzene, toluene, *o*-xylene	rhizosphere effect
Little bluestem (*Schizchyrium scoparious*	chrysene, benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a,h*]anthracene	unknown
Indiangrass (*Sorghastrum nutans*)	chrysene, benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a,h*]anthracene	unknown

Rhizosphere Effects on Soil Contaminants

Growth Linked Metabolism

Selective Enrichment

Cometabolism

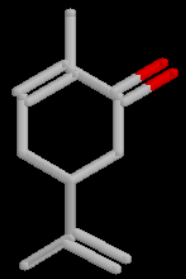
Surfactants

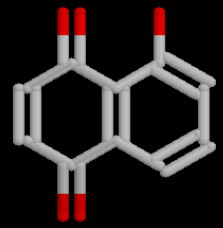
Selective Enrichment:

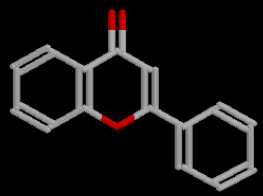
Diversity of PAH degrading bacteria in Esturarine Grasses (Daane et al., 2001)

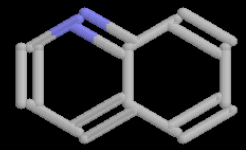
Plant species			
Distichlis spicata	*Juncus gerardi*	*Phragmites australis*	*Spartina alterniflora*
Paenibacillus validus	*Paenibacillus validus*	No PAH degraders	*Paenibacillus validus* *Pseudomonas stutzeri* *Pseudomonas putida* *Rhodococcus ruber* *Tsukamurella wratislaviensis* *Arthrobacter oxydans* *Sphingomonas subarctica*

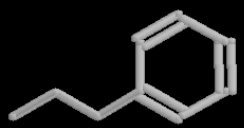
Co-Metabolism
Phytochemical Analogs of Xenobiotic Contaminants

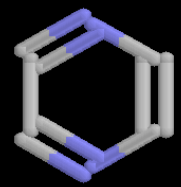
Terpenes: 

Quinones 

Flavonoids 

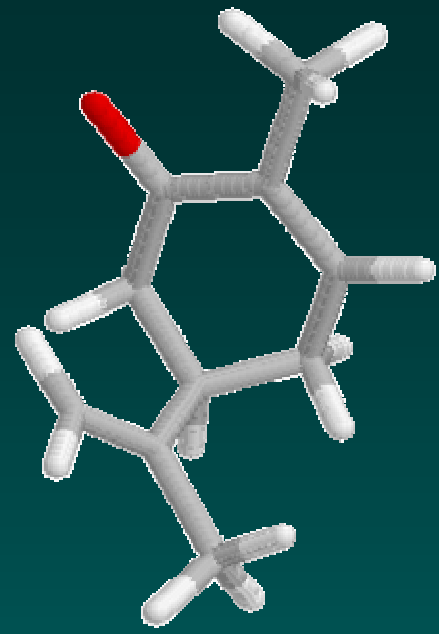
Alkaloids 

Lignin 

Pyrazines 

Plant Screening Assay

Gilbert and Crowley 1996



Spearmint (*Mentha spicata*)

Active component: carvone

Arthrobacter B1B

Evidence:

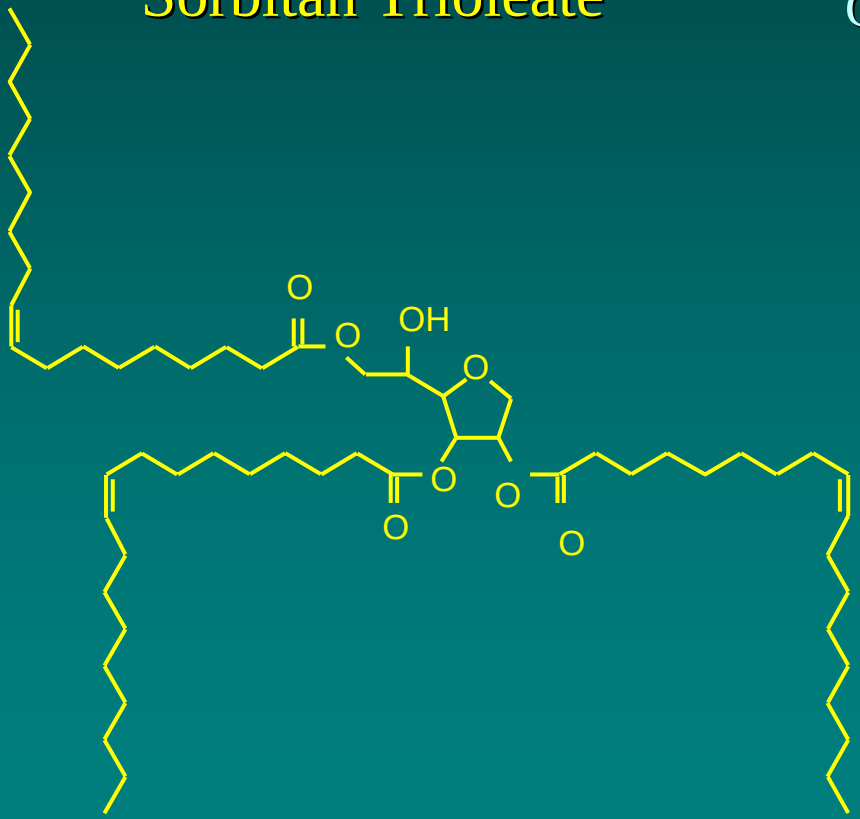
PCB ring oxidation product

chlorobenzoate product formation

disappearance of Aroclor 1242

Development of a Field Application Vector

Sorbitan Trioleate



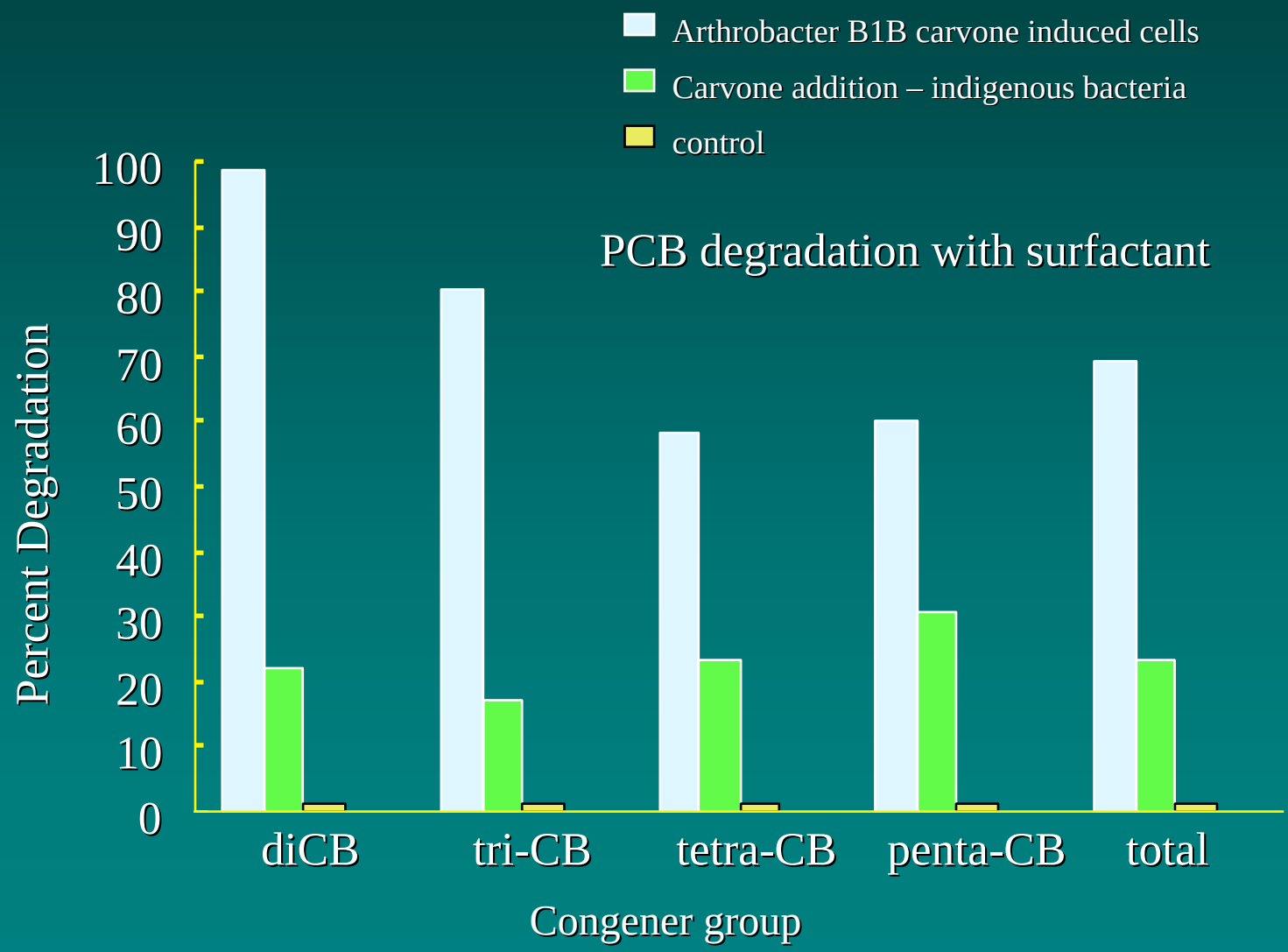
Criteria:

- Nontoxic
- Selective Growth Substrate
- Solubilizes PCB
- Biodegradable

Fatty Acid Composition:

Oleic acid	**74%**
Linoleic acid	**7%**
Linolenic acid	**2%**
Palmitoleic acid	**7%**
Palmitic acid	**10%**

Cometabolic Degradation of Aroclor 1242 PCBs



Salicylate: Plant Signal Compound, Siderophore, and Inducer of Xenobiotic Degradation Enzymes

Toluene dioxygenase substrates:

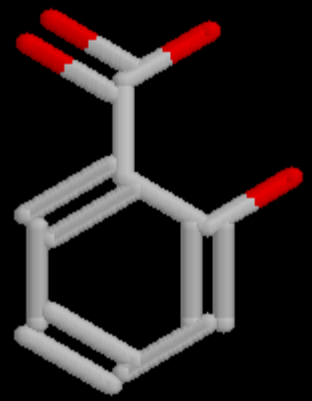
Benzene, toluene, xylene (BTEX)

Trichloroethylene (TCE)

Trinitrotoluene (TNT)

Naphthalene, benzopyrene (PAH)

Polychlorinated biphenyls (PCB)



Chenopodium ambrosioides

Alpha-pinene, Aritasone, Ascaridole, Ascorbic-acid, Beta-carotene, Butyric-acid, Calcium, D-camphor, EO, Ferulic-acid, Geraniol, L-pinocarvone, Leucine, Limonene, Malic-acid, Menthadiene, Methyl-salicylate, Myrcene, Niacin, P-cymene, P-cymol, Phosphorus, Safrole, Saponins, Spinasterol, Tartaric-acid, Terpinene, Terpinyl-acetate, Terpinyl-salicylate, Thiamin, Triacontyl-alcohol, Trimethylamine, Urease, Vanillic-acid

Phytoremediation of Pyrene using Celery Root

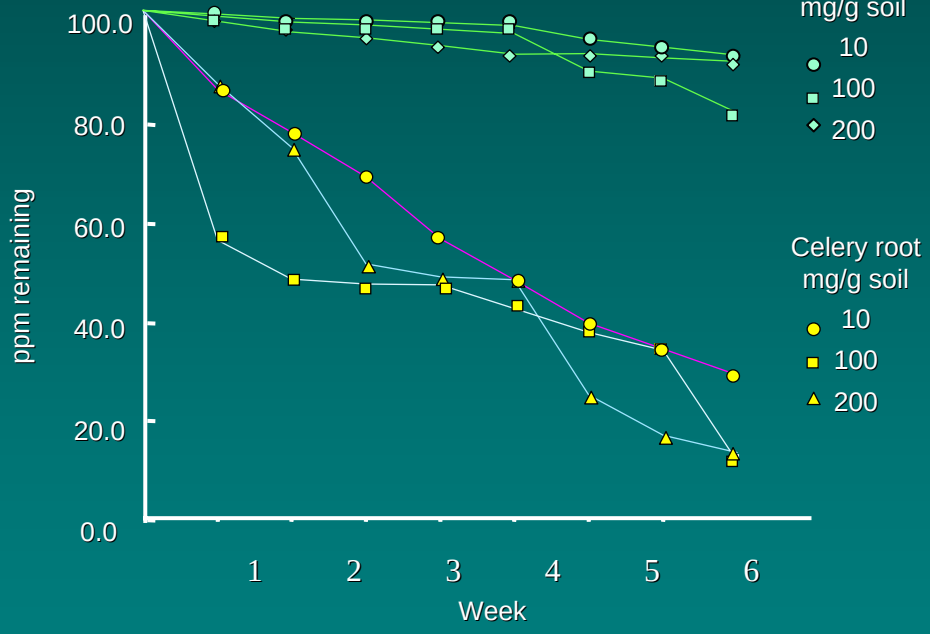
Root essential oil:

	ppm
β pinene	15,000
carvone	5000
dihydro-carvone	5000
p-cymene	31,000
limonene	117,000
myrcene	18,000
terpenoline	33,000
trans-ocimine	290,000
cis-ocimine	68,000

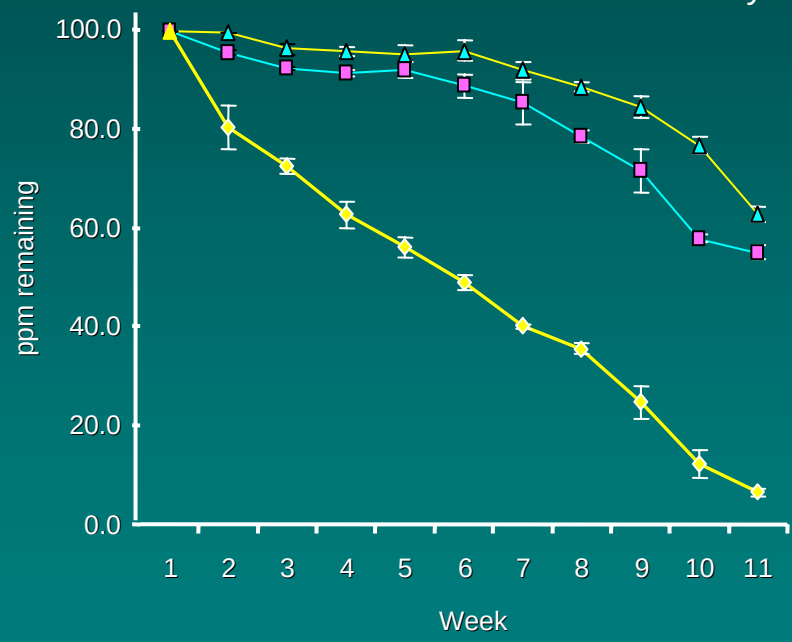
Duke, J. A. 1992. Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL. CRC Press.

Degradation of pyrene in soil amended with pulverized celery root or in the rhizosphere of wheat and celery plants

Organic amendment



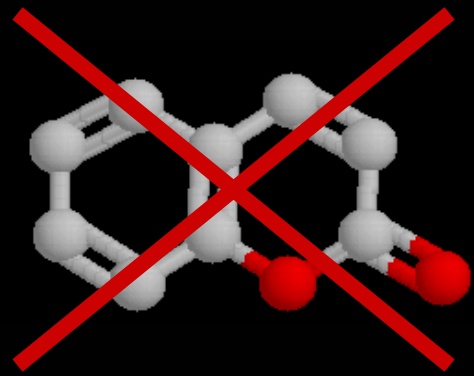
Rhizoremediation



What is the active inducing substance in celery?

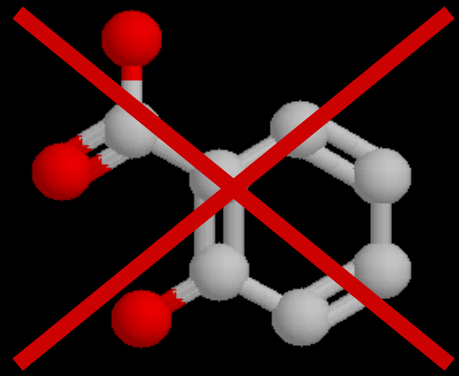
Terpenes

Coumarin



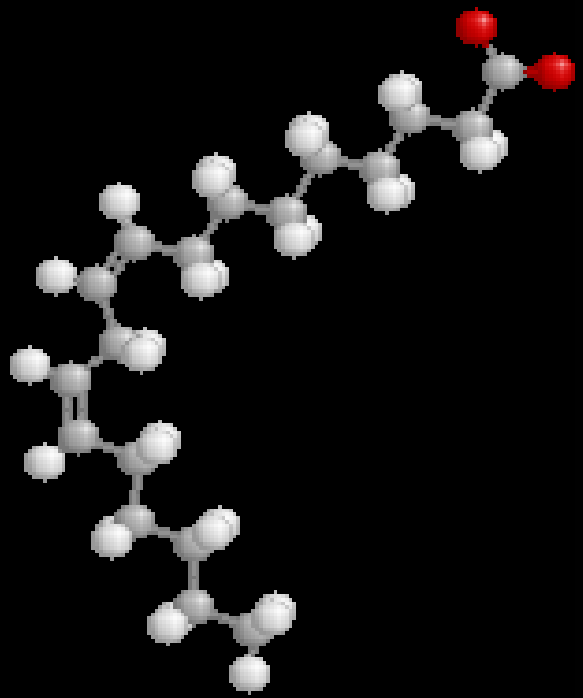
Coumaric acid

Salicylic acid



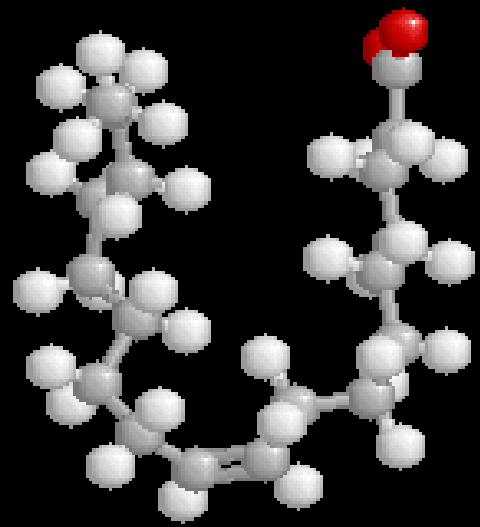
Linoleic Acid

$C_{18}H_{32}O_2$



Oleic Acid

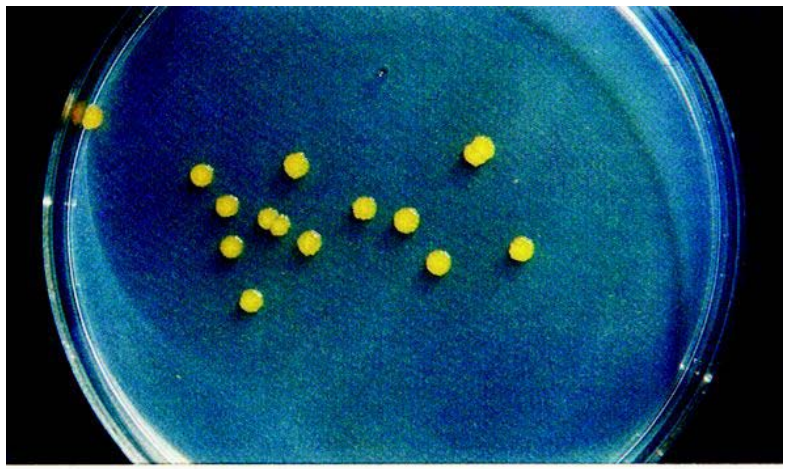
$C_{18}H_{34}O_2$



Biosurfactants from Rhizosphere Microorganisms

Pseudomonads	Rhamnolipids
Sphingomonas	Sphingans
Mycobacterium	Extracellular polymers
Plants	Saponins, Linoleic Acid
Fungi	Linoleic acid

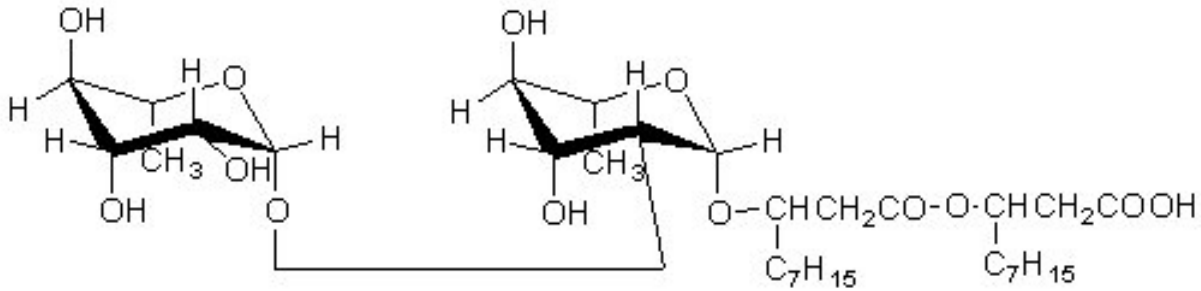
Mycobacteria



Sphingomonas sp.

Rhamnolipid

Pseudomonas aeruginosa



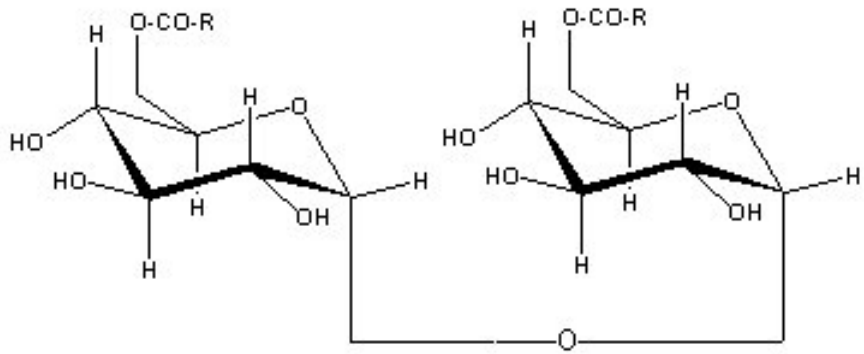
Acylated glycosides

Rhodococcus

Mycobacterium

Nocardia

R = palmitic acid, C16:0; oleic acid, C18:1n-9; linoleic acid, 18:2n-6

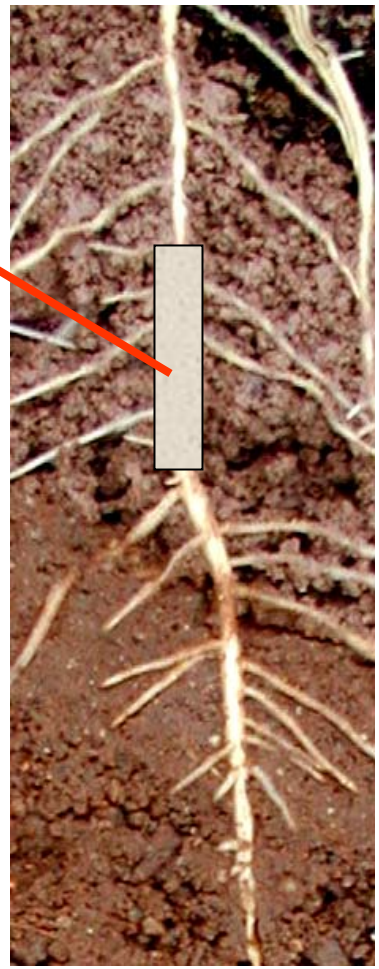


6,6'-diacyltrehalose

R-CO : palmitoyl or mycoloyl moiety

Microsite sampling methods for comprehensive analysis of pollutant degrading communities.

Pollutant impregnated quartz filter strips

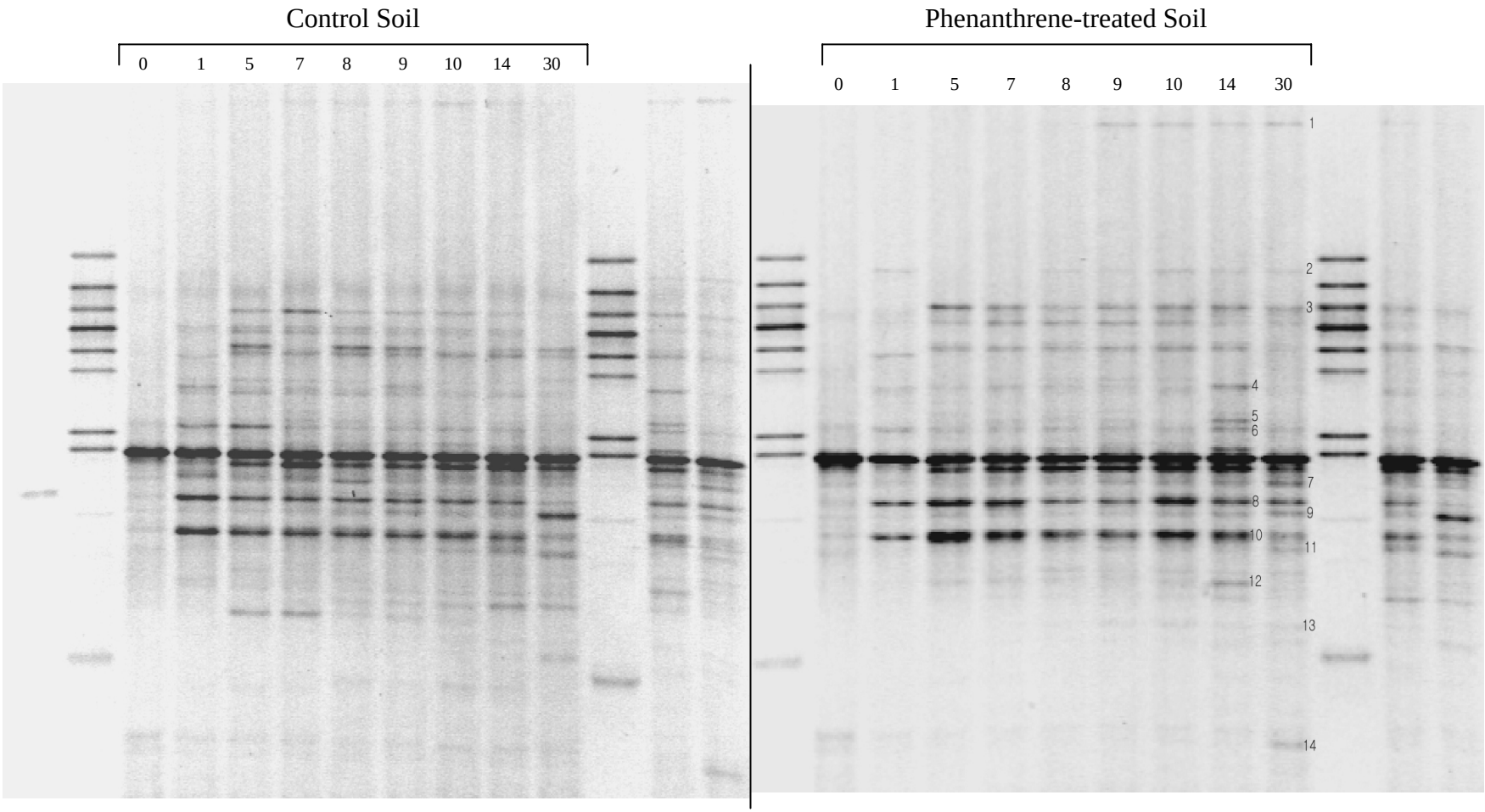


Degradation / solubilization assays

Microbial community analyses

PCR-DGGE
Real time PCR
Clone Libraries
Gene Probes
Reporter Gene Assays

Phenanthrene Treatment and Bacterial Community Shift



Research Needs

- Determine the importance of plant and microbially produced surfactants for enhancing bioavailablity of metal and organic pollutants in the rhizosphere.

- Identification of bacterial species and genes that function in soils for biodegradation of PCBs and PAHs.

- Establish the relevance of microbial consortia: eg. surfactants, cooperative, sequential degradation pathways.

Conclusions

- Enhanced degradation of xenobiotics in the rhizosphere can be faciliated by several processes that function alon or in combination. These include selective enrichment of degraders, growth-linked metabolism, release of cometabolic substances, and production of surfactants.

- Biosurfactants appear to have a key role in the bioremediation of both metal and organic pollutants, but too date have been almost completely ignored in research on rhizoremediation.

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Innovation in life sciences

Rhamnolipid - Producing Plants

Phytoremediation of oil and heavy-metal contaminated soils

Plants with enhanced contamination tolerance and disease resistance

Researchers at the Sainsbury Laboratory (Norwich, UK), in collaboration with the Institute of Genetics and Cytology (Minsk, Belarus), have successfully produced rhamnolipids in transgenic plants expressing genes derived from the soil bacterium *Pseudomonas aeruginosa*.

Evaluation and Licensing Opportunities

For further information on this technology and evaluation/ licensing opportunities please contact:

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Tech ID: 01.287

Patent Literature

International Patent Publication No. WO 2004/050882