Studio of a bacterial consortium adapted to low bioavailability to phenanthrene, as potential inoculant to chronically PAH contaminated soils

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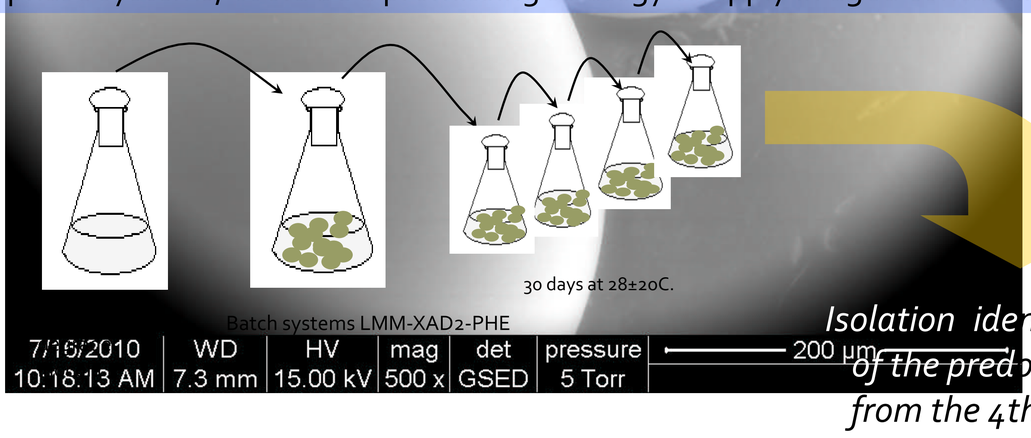
Introduction

Polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants produced by industrial operations using fossil fuels as well as by natural events such as forest fires. Microorganisms are the major agents mineralizing PAH in terrestrial environments. PAHs have a strong tendency to adsorb onto solid surfaces, especially hydrophobic sites.
The establishment of microniches of PAH degrading bacteria in contaminated soil is a process influenced by the manner in which the PAH are exposure in the soil and bacterial capability to develop strategies to adapt to different PAH bioavailabilities.
In order to reduce phenanthrene bioavailability to different degrees, Grosser and coworkers, 2000, proposed the establishment of enrichment cultures in which solid organic phases were used.
Due to the reduced bioavailability that characterizes chronically hydrocarbons contaminated soils, the bioaugmentation with cultures obtained by enrichment in solid phase systems, could be a promissing strategy to apply in aged contaminated soils.



Isolation identification and study of the predominant sessil cells from the 4th LMM-XAD2-PHE system.

Objective

Study of four bacterial components obtained from a degrader phenanthrene consortium by using solid phases with a resin XAD2 preloaded with phenanthrene, and the defined degrading consortium rebuilt from them.

Materials and Methods

Phenanthrene degrader inocula: Enrichment culture of phenanthrene degrading bacteria in liquid mineral medium (LMM) by using a petrochemical contaminated soil (10% w/v) and 0.1g of phenanthrene cristals l-1.

Bacterial consortium: Enrichments in Amberlite® XAD®-2 preloaded with phenanthrene (10.87mg of phenanthrene g-1 XAD2).
The batch system LMM-XAD2-PHE was inoculated with 1ml of phenanthrene enrichment culture in 20ml of LMM containing 2g of XAD2 preloaded with phenanthrene (1000ppm).
A 10% of colonized solid phase was removed, washed and transferred to fresh systems. As a whole, four transfers were done.

Results and discussion

Table 1: Physiological properties of the strains.

Strains	C1	C2	C3	C4
Cell Morphology	Regular rods	Regular rods	Rods	Cocci-rod cycle
Colony morphotype	Small, yellow	Small, extended, transparent	Pink, opaque	White, extended, mucous
Gramreaction	Gram-negative	Gram-negative	Gram-positive	Gram-positive
Motility	+	-	-	-
OF - glucose	O [a]	inert	O [a]	O [a]
Hemolytic activity	-	+	-	-
Cytochrome oxidase	+	-	-	-
Indigo production	+	-	-	-
Siderophores	-	+	+	-
Phosphorus solubilization	+	-	-	+
R3 broth (µ, hs^{-1})	0.38 ± 0,07	0,23 ± 0,01	0,20 ± 0,05	0,50 ± 0,05
LMM-D-glucose (µ, hs^{-1})	0.14±0.03	No growth	0.21±0.01	0.22±0.04
LMM-PHE (µ, hs^{-1})	0.12±0.05	No growth	No growth	0.05±0.01
SMM-PHE	halo	halo [b]	Growth without halo	halo

[a] Mild acidification
[b] The initial ability to produce a solubilization halo was lost through successive passages on SMM-PHE.

Table 2: Results of 16s rRNA gene sequence and phylogenetic affiliations of the strains isolated by solid phase model with resin XAD 2 preloaded with phenanthrene.

Strain	Most closely related bacterial 16S rRNA gene sequence	Cover[a]	Max identity	Accesion number [b]
C1	*Sphingobium fuliginis strain TKP*	1324/1485	99	DQ092757.1
C2	*Acidovorax avenae*	1285/1285	99	AY512827.1
C3	*Rhodococcus opacus strain ML0004*	1351/1485	99	DQ474758.1
C4	*Arthrobacter globiformis strain A2S3*	1354/1485	99	EU221407.1

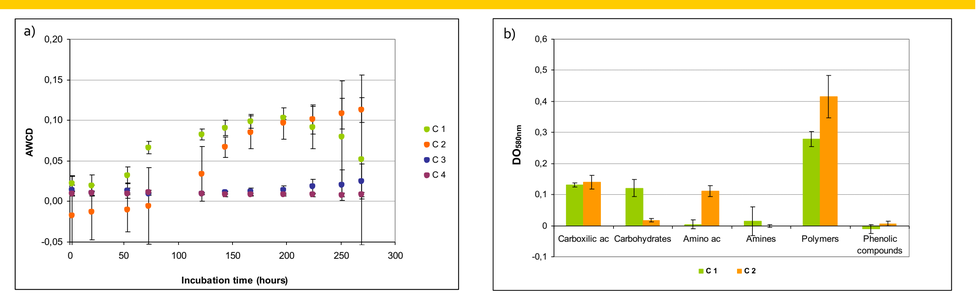


Figure 1: Average well color development (AWCD) of substrate utilization profiles in Biolog EcoPlate™ microplates from the isolated strains (a). Total utilization of carbon substrates from different substrate groups by C1 and C2 strain, at 200hs of incubation (b).

The presence of these properties in the defined consortium could be interesting at the moment to propose apply it in a nutrient deficient soils, as the chronically hydrocarbon contaminated soils are.

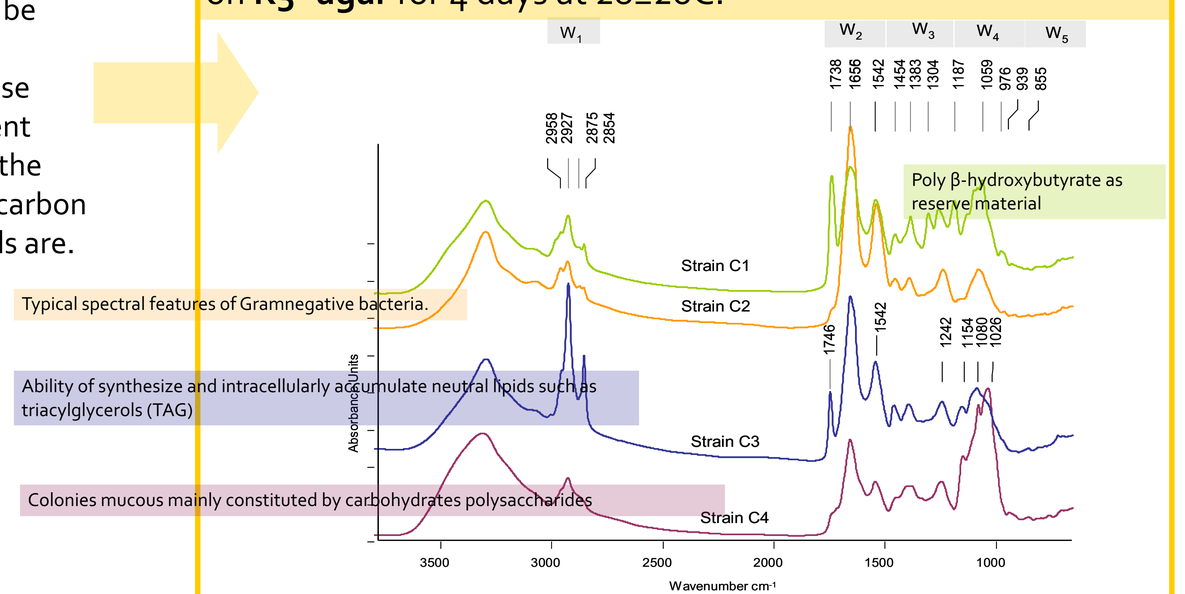
Growth of the four individual strains growing in LMM-XAD2-PHE system.

Table 3: Bacterial growth on the resine beads preload with phenantrene XAD2-PHE and on XAD, in LMM batch systems.

System		XAD2 (Ufc of sessil cells g-1)		XAD-PHE (Ufc of sessil cells g-1)	
Incubation time		10 days	30 days	10 days	30 days
Strains	C1	[a] $5.13.10^7$	[bc] $1.97.10^6$	[c] $9.52.10^5$	[b] $4.21.10^6$
	C2	[b] $4.21.10^6$	[a] $7.73.10^5$	[a] $1.22.10^6$	[a] $2.81.10^5$
	C3	[b] $4.21.10^5$	[a,b] $1.36.10^6$	[b] $9.63.10^5$	[a] $7.65.10^6$
	C4	[a] $1.37.10^7$	[b,c] $4.90.10^5$	[a,b] $6.61.10^6$	[c] $1.32.10^5$

Values, gives as means of triplicate independent experiments with the same letter are not significantly different (P< 0.01).

FT-IR spectra analysis of the four individual strains growing on **R3 -agar** for 4 days at 28±20C.



Re-building a defined degrading consortium:
C1 + C2 +C3 +C4

In this culture condition the strains grew more significantly adsorbed to XAD-PHE beads during all incubation time.

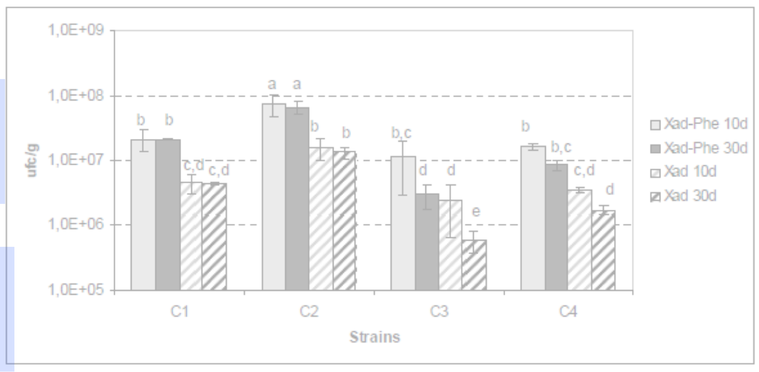


Figure 3: Growth of the sessil cells after 10 and 30 days in the model organic phases, with XAD2 and XAD2 preloaded with phenanthrene. The different cell morphotypes coud be distinguished and plate counted during the incubation of the defined consortium in LMM-XAD and LMM-XAD-PHE. Values, gives as means of triplicate independent experiments with the same letter are not significantly different (P< 0.01).

Table 4: Phenanthrene remaining and metabolic intermediates from the strain pure cultures and the defined consortium in LMM-XAD2-PHE, after 30 days of incubation.

	Phenanthrene elimination (%)	1-Hydroxy-2-naphthoic acid [1] (%)	Salicylic acid[1] (%)
C1	60.5 ± 1.6 a,b	85.5 ± 23.3 a	0.19 ± 0.13 a
C2	19.9 ± 11.1 c	13.8 ± 10.3 b	Nd
C3	24.4 ± 5.4 c	38.8 ± 1.0 a	Nd
C4	47.1 ± 7.8 b,c	17.0 ± 0.3 b	Nd
Consortium	69.7 ± 3.2 a	Nd	0.04 ± 0.02 a
Abiotic Control	7 ± 3.1 d	-	-

Nd: no detected
[1]The hydroxi naphtoico and salicilic acids values represent the percent of the metabolite of the total eliminated phenanthrene. Value, gives as means of triplicate independent experiments with the same letter are not significantly different (P< 0.01).

Conclusion

- Interesting properties of each strain were detected, as phenanthrene degrading capacity in pure culture; ability to produce and accumulate a significant amount of reserve material like as PHB or TAG; abundant extracellular polysaccharides production; siderophore production and phosphorus solubilization and capacity to adherence to hydrophobic surfaces.

- When the strains were cultured toghether in the defined consortium with the phenanthrene adsorbed to XAD2, the degrading capacity was significantly higher than in the pure culture of its constituent members.

- The consortium growth in the biofilm increased the phenanthrene degrading capacity and promoted the individual strains growth. This synergic relationship between the strains, possible due to growth factors or metabolic intermediates, could explain the higher phenanthrene-removal efficiency of the consortium, with no significant intermediates accumulation.