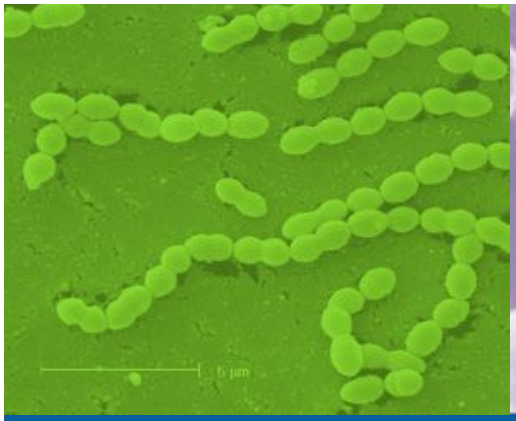

Isolation, characterization and application of soil microorganisms useful for the degradation of recalcitrant pollutants and for the stimulation of plant growth

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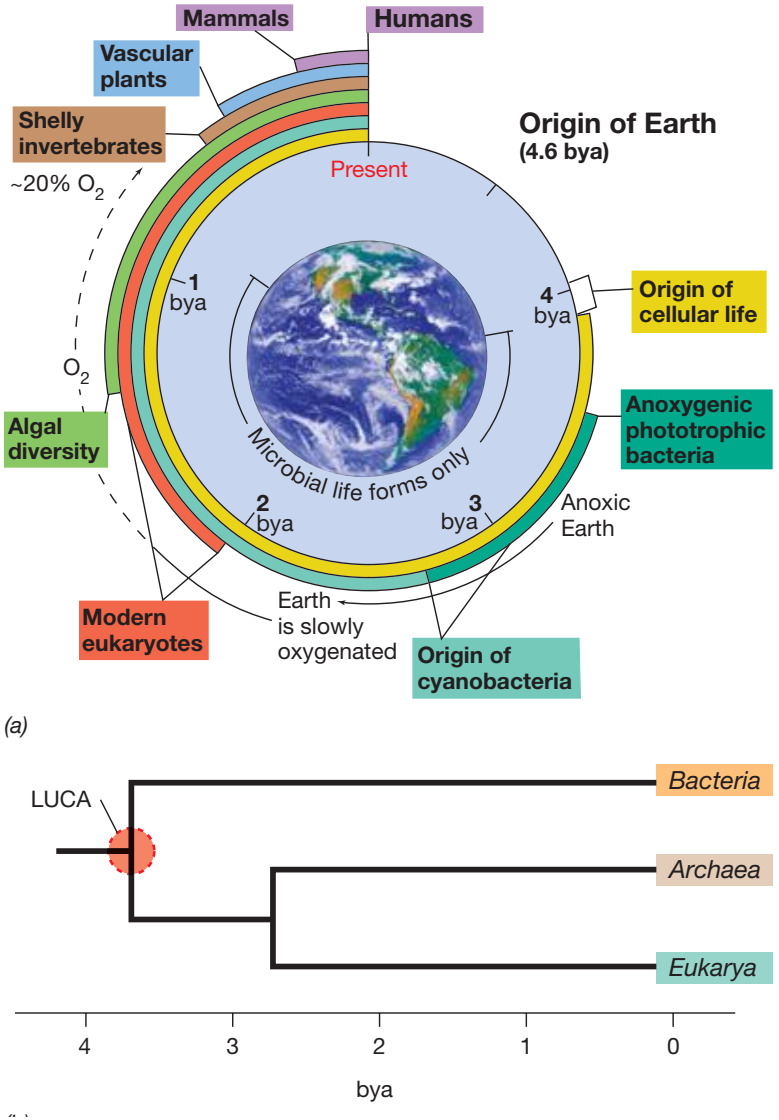
GENERAL FEATURES OF BACTERIA

- Oldest organisms on Earth
- Unicellular, prokaryotic, small
- Highest surface/volume ratio
- Highest diversity on Earth
- Simple yet extremely versatile ("small but not stupid")
- Infinite and constant adaptation to new conditions (xenobiosis is hence a momentary condition)
- All possible metabolisms are represented in bacteria



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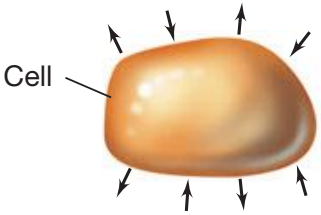
BACTERIA

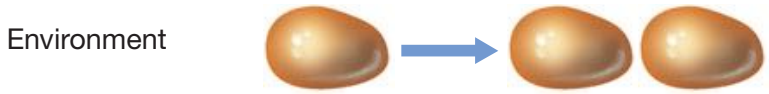
I. Properties of all cells

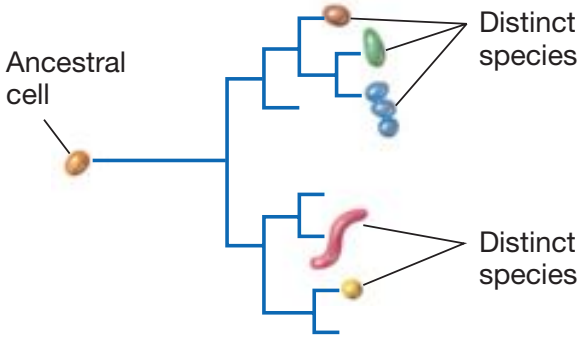
Compartmentalization and metabolism
A cell is a compartment that takes up nutrients from the environment, transforms them, and releases wastes into the environment. The cell is thus an *open* system.

Growth
Chemicals from the environment are turned into new cells under the genetic direction of preexisting cells.

Evolution
Cells contain genes and *evolve* to display new biological properties. Phylogenetic trees show the evolutionary relationships between cells.





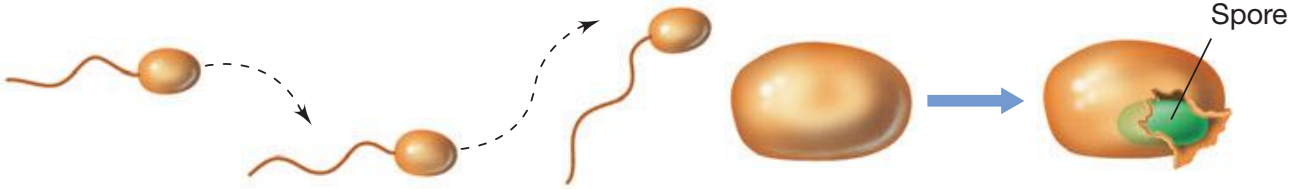


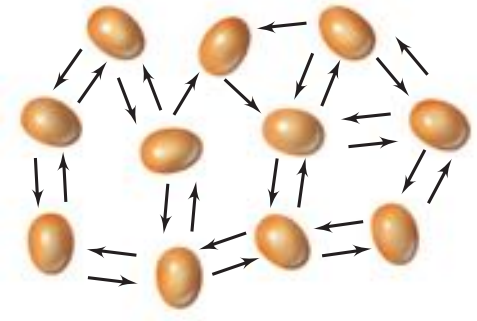
II. Properties of some cells

Motility
Some cells are capable of self-propulsion.

Differentiation
Some cells can form new cell structures such as a spore, usually as part of a cellular life cycle.

Communication
Many cells *communicate* or *interact* by means of chemicals that are released or taken up.





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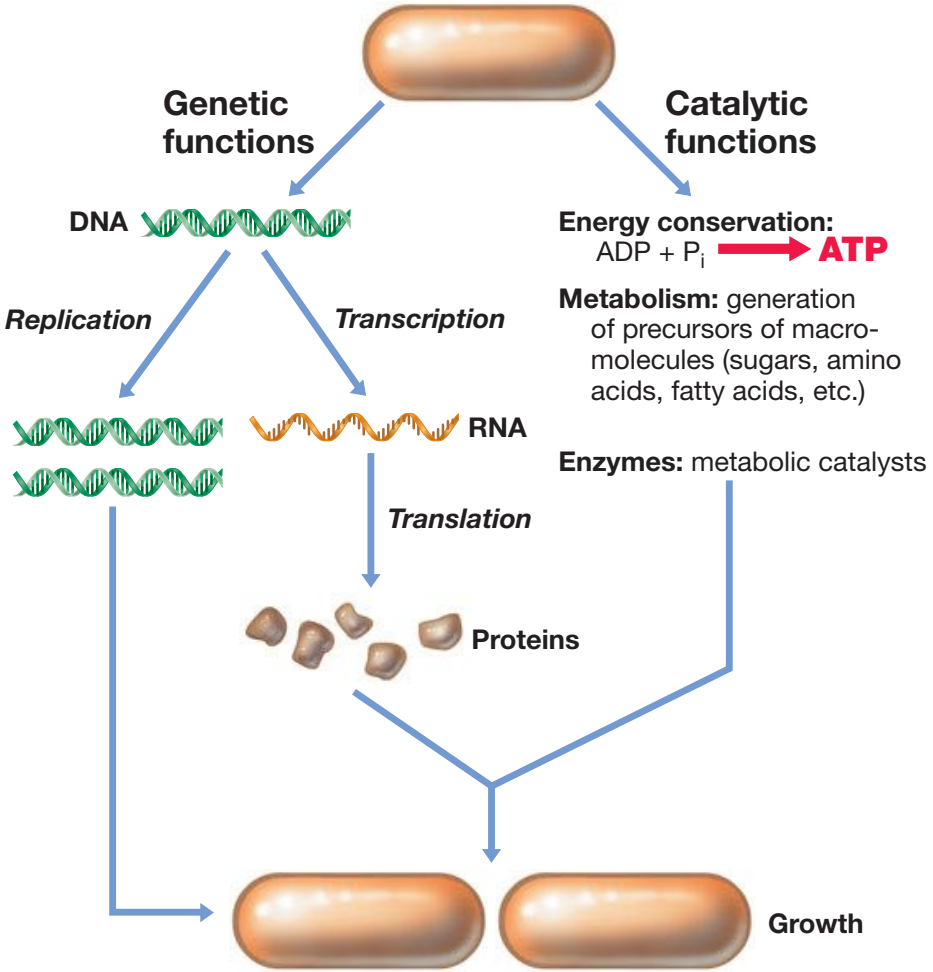
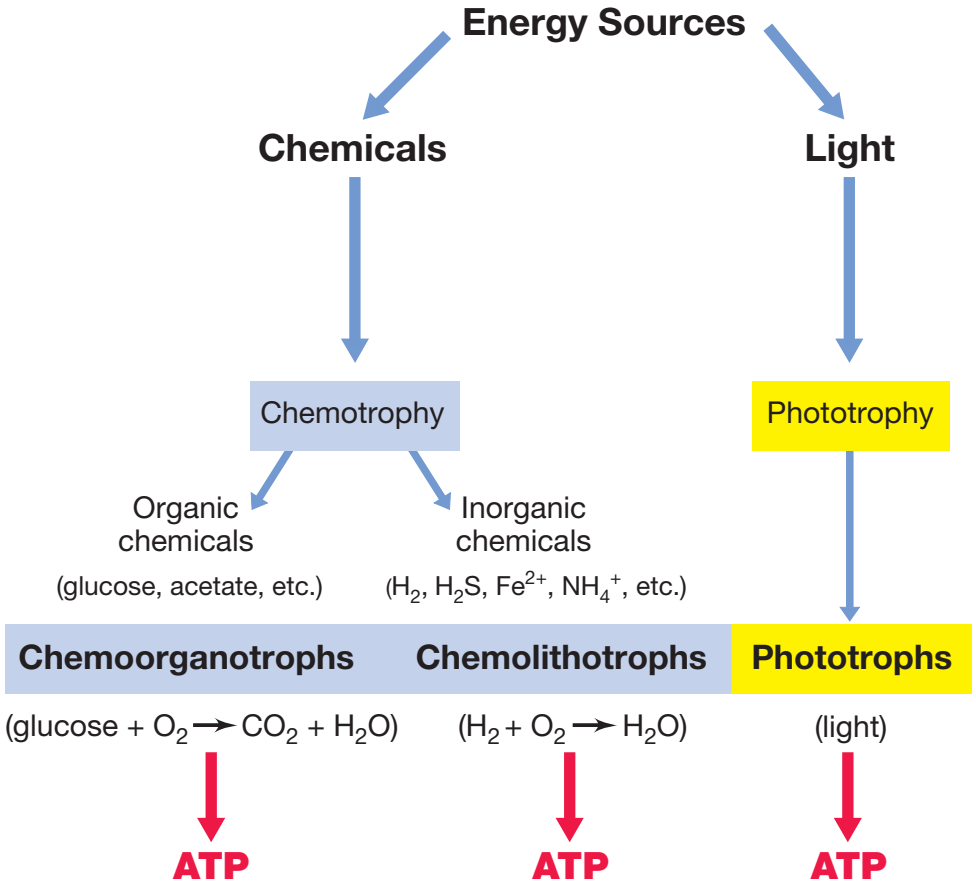
BACTERIA





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BACTERIA IN SOILS

Processes depending on direct or indirect MO activity

OM decomposition

Nutrients cycling

Nutrients release

Atmospheric N_2 fixation

Suppresions of plants diseases

Soil structure

Pollutants degradation and mitigation

Sink and source of GHG

How many they are?

microbial biomass in 1ha of soils = a cow!

Up to 1 milion of species in a gram of soil

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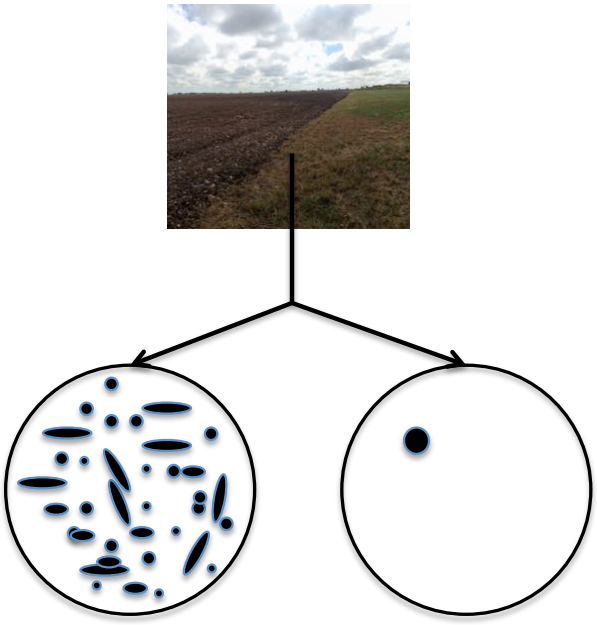
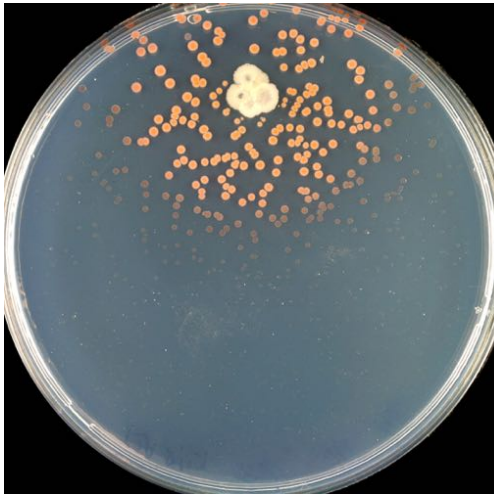
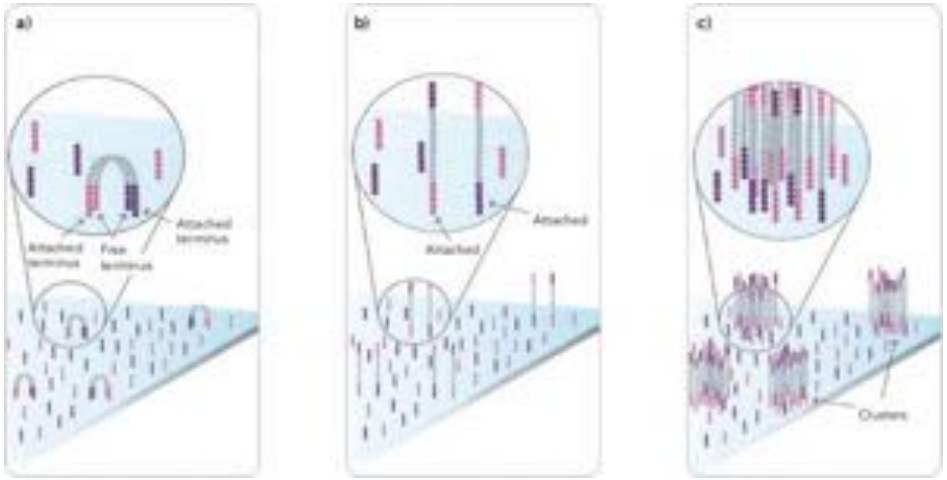
THE GREAT PLATE COUNT ANOMALY



- Most soil microorganism are not cultivable
- We can address this “unculturable majority with molecular methods
- Next generation sequencing methods are giving us the possibility to ”virtually” analyse all microorganisms that are present in a given environmen





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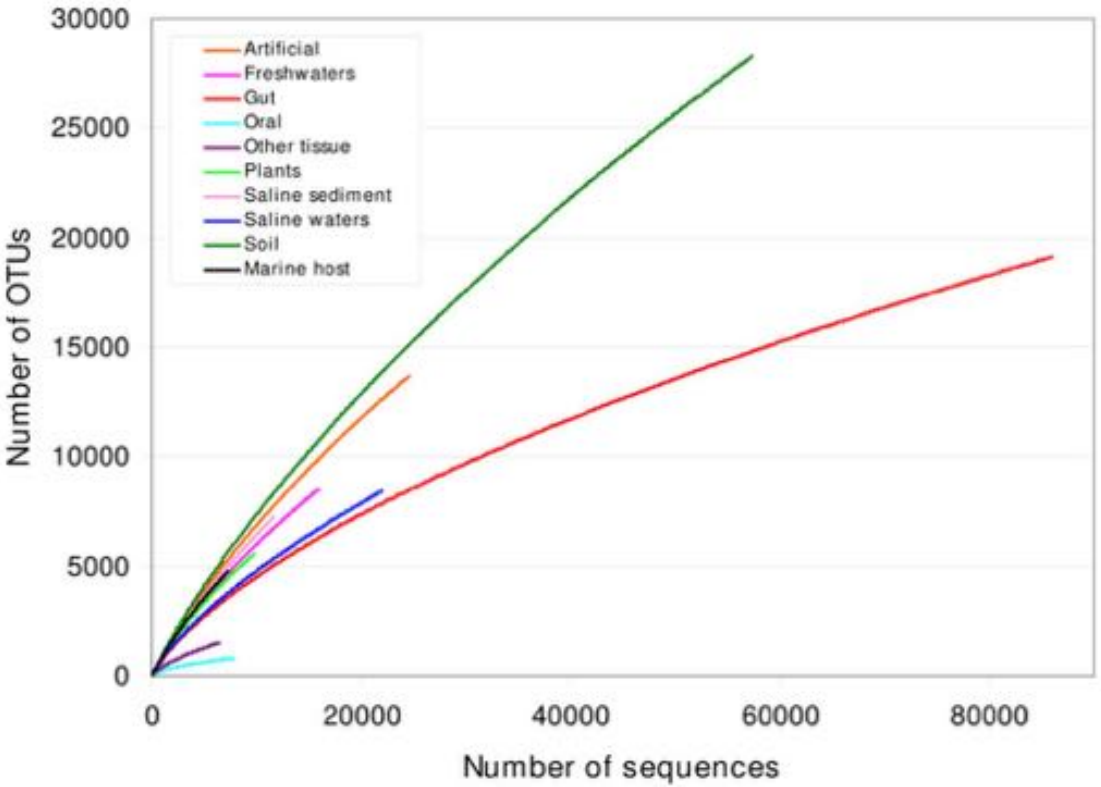
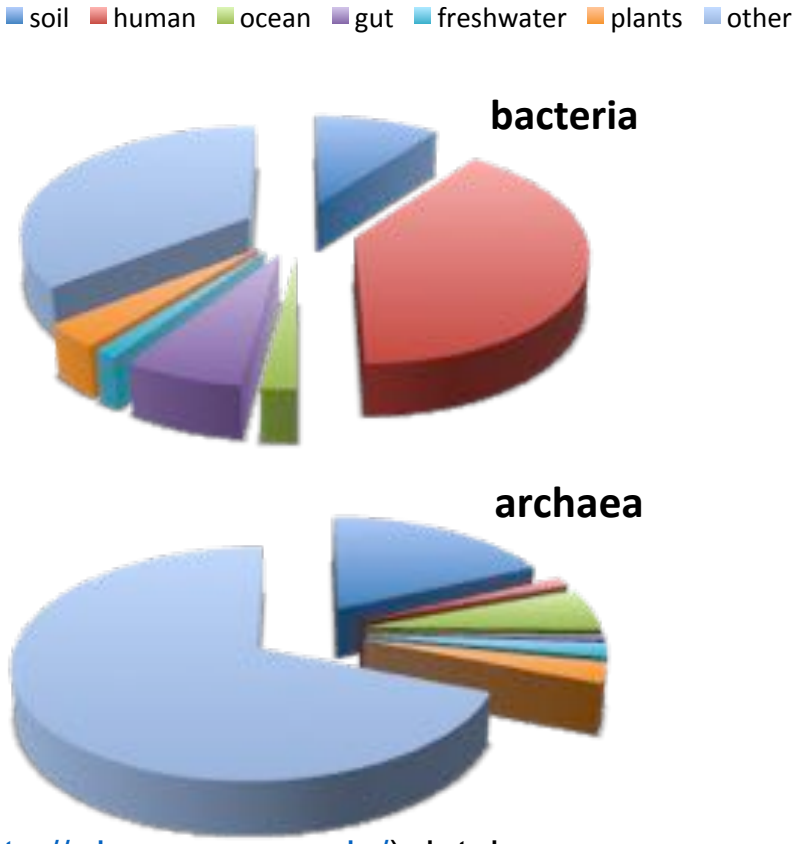
PROKARYOTIC DIVERSITY IN SOIL vs OTHER ENVIRONMENTS



Tamames et al. *BMC Microbiology* 2010, **10**:85



RDP (http://rdp.cme.msu.edu/) database query among 1727996 (bacteria) and 73354 (archea) total sequences

N° of 16S sequences in RDP does not reflect prokaryotic environmental distribution and abundance. Soil is most challenging

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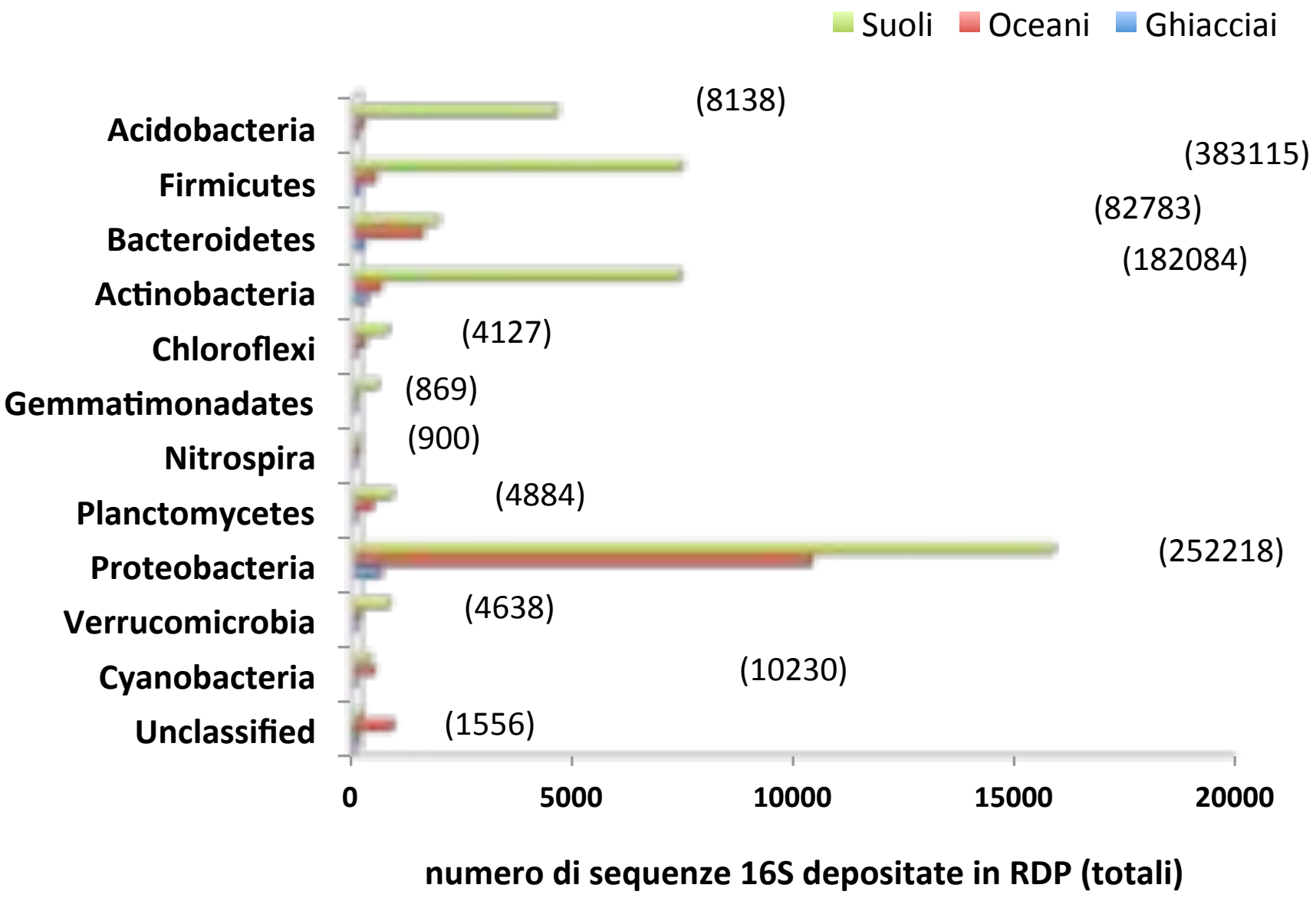
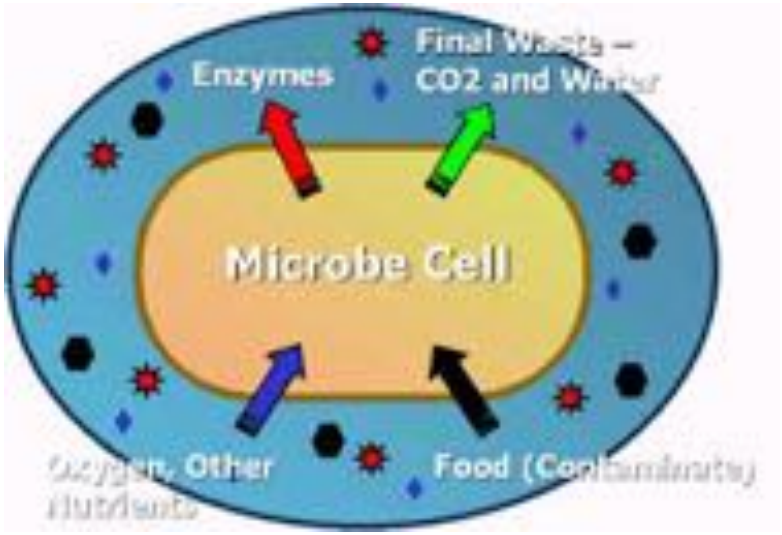
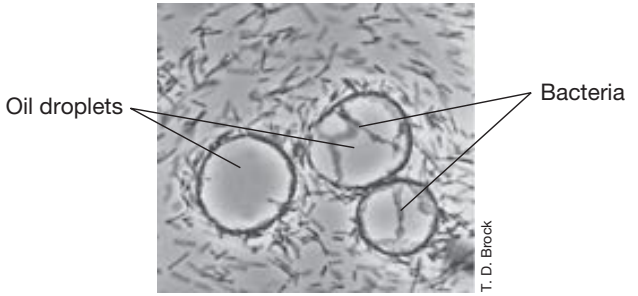
PROKARYOTIC DIVERSITY IN SOILS: WHO IS THERE?



BACTERIA AND BIOREMEDIATION





- **Xenobiotic:** a chemical compound that is foreign to a living organism
- **Mineralization**: contaminants are used as C source and completely metabolized;
- **Co-metabolism**: contaminants are not used as C source; they are transformed at different levels.
- **Immobilization / partitioning**: pollutants' bioavailability is reduced through adsorption, accumulation and/or precipitation phenomena.

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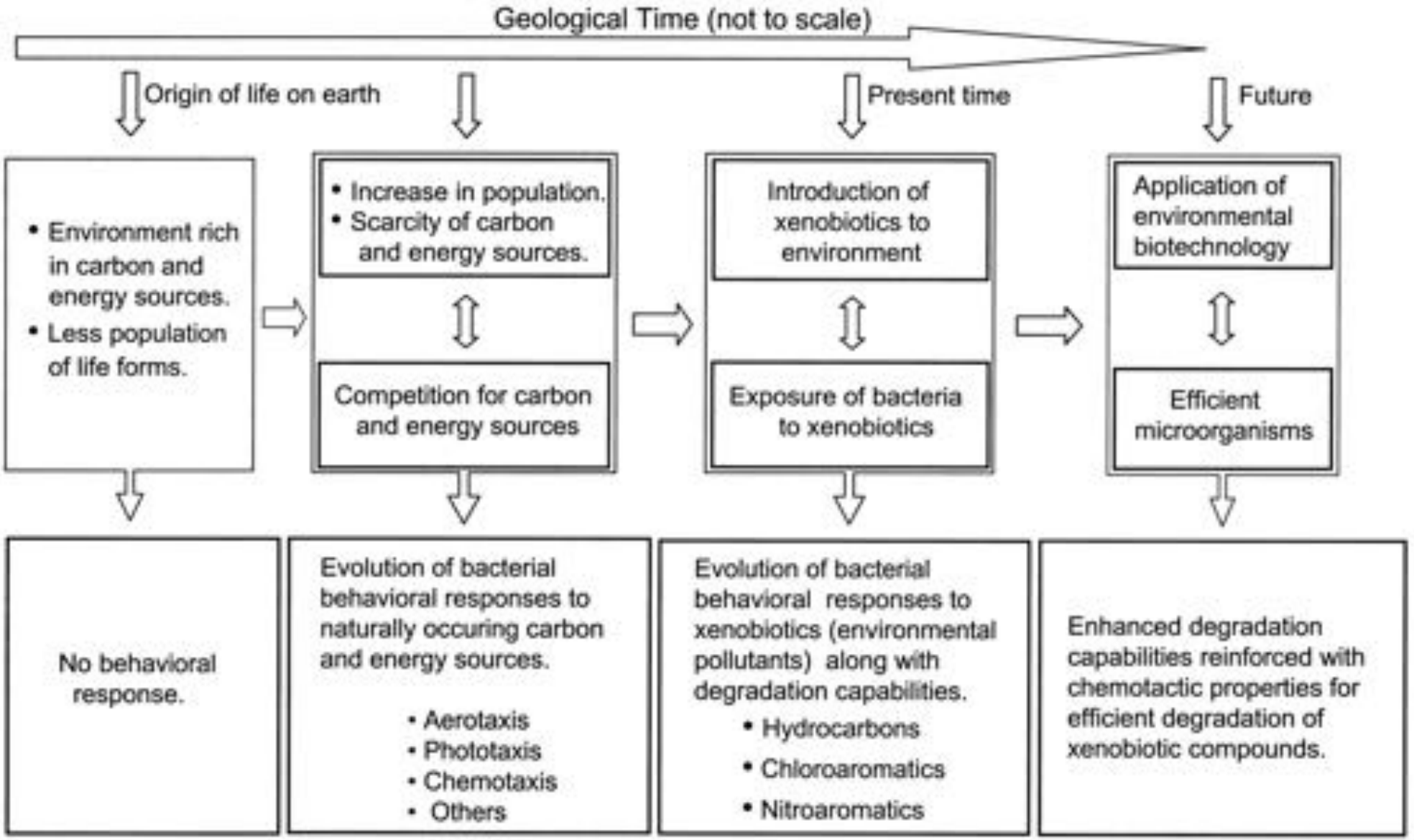
XENOBIOTICS AND MICROBES



Pandei & Jain **Bacterial chemotaxis towards environmental pollutants: role in bioremediation**. *AEM* 68, 5789-5795 (**2003**)

Table 1
Degrading activity of some simple bacteria, and in mixture [1,12].

Organism	PAH degradation (%), standard deviation it is ≤3%				
	Naphthalene	Acenaphthene	Fluorene	Anthracene	Pyrene
Pseudomonas sp.	15.5	28.0	24.4	25.4	92.3
Pycnoporus sanguineus	12.0	7.0	17.6	15.6	4.4
Coriolus versicolor	27.4	2.0	23.0	22.4	42.0
Pleurotus ostreatus	29.4	20.6	20.6	19.0	32.0
Fomitopsis palustris	19.5	7.5	7.0	31.7	7.3
Daedalea elegans	35.8	5.9	5.9	2.4	26.1
Pycnoporus sanguineus mixed with *Pseudomonas* sp.	13.5	29	24.2	11.4	17.4
Coriolus versicolor mixed with *Pseudomonas* sp.	15.5	27	24	25.0	93.7
Pleurotus ostreatus mixed with *Pseudomonas* sp.	13	25	19	20.0	17.0
Fomitopsis palustris mixed with *Pseudomonas* sp.	13.1	16.3	16.3	12.0	93.7
Daedalea elegan mixed with *Pseudomonas* sp.	23	14.9	14.9	3.4	46.4
Aerated soil at 40% WHC in presence of *Sphingomonas* and *Azospirillum*		100	100	84	87
Aerated soil at 40% WHC; KNO_3 and K_2HPO_4 in presence of *Sphingomonas* and *Azospirillum*		100	100	81	90
Aerated soil at 40% WHC; nutrients: biosurfactant MAT10		100	100	79	90
Aerated soil at 40% WHC; nutrients; ferric ion added as ferric octoate		100	100	87	88

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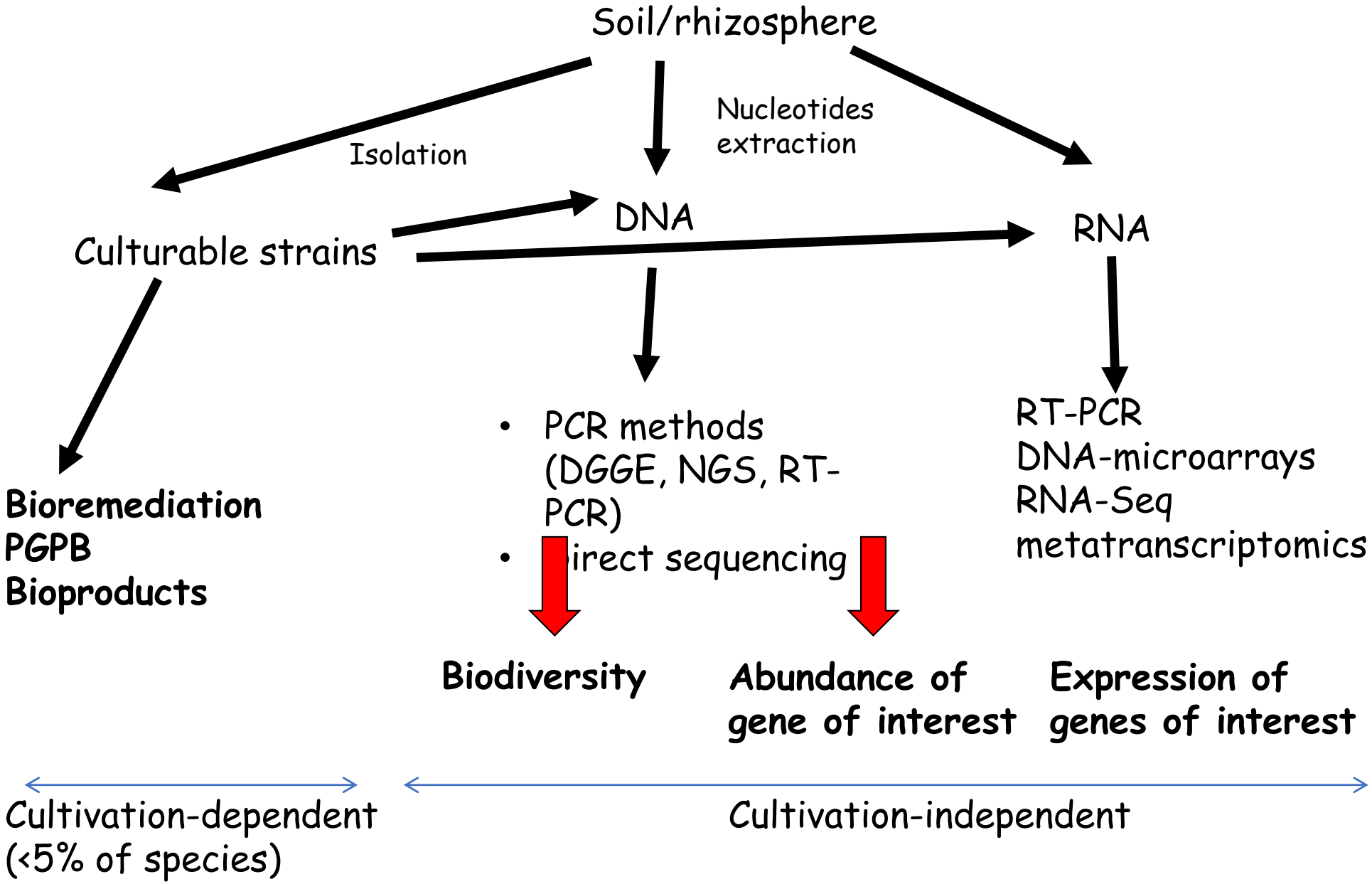
EXPERIMENENTAL APPROACHES FOR SOIL MICROBIOLOGY STUDIES



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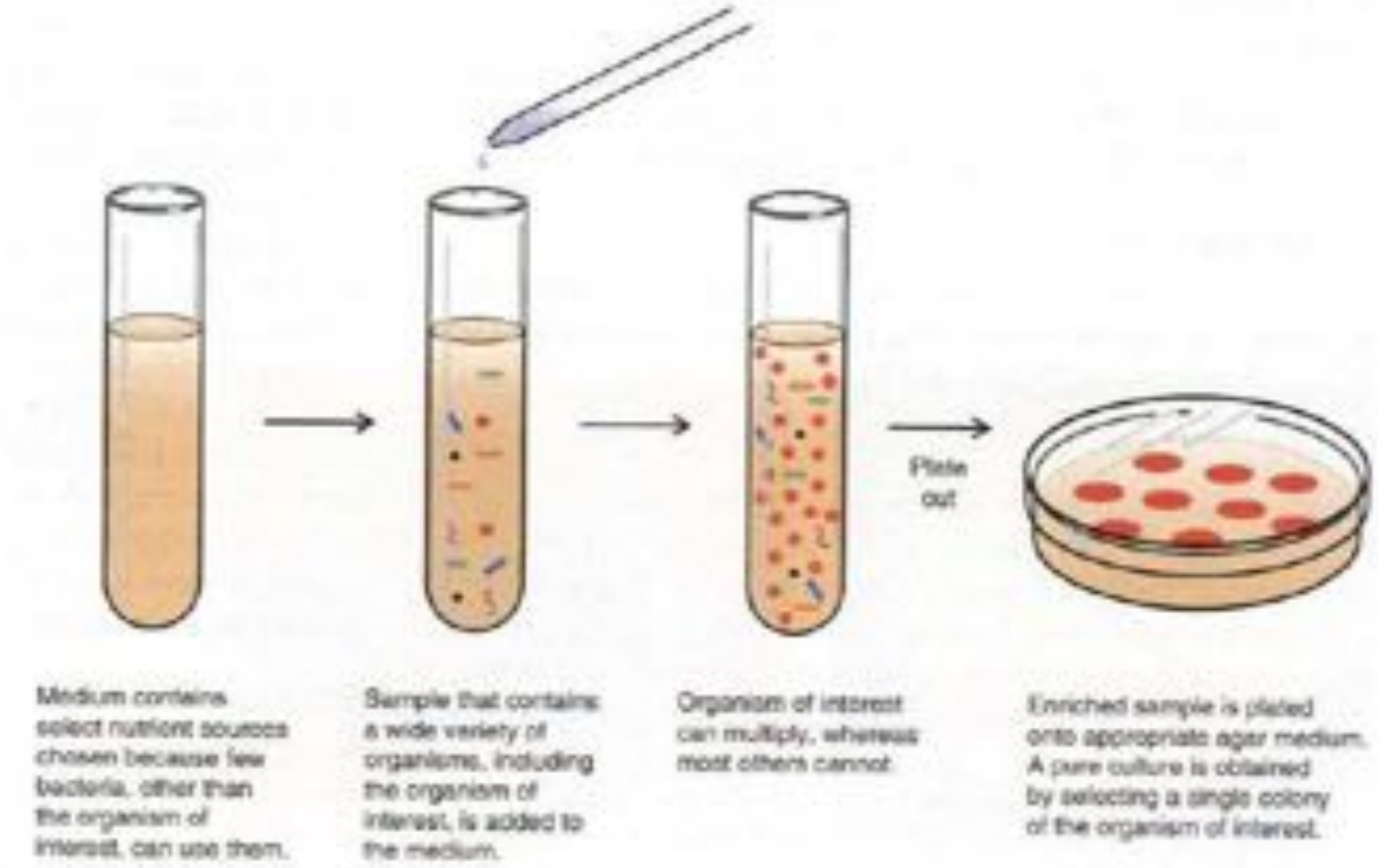
CULTURE-BASED METHODS: ENRICHMENT



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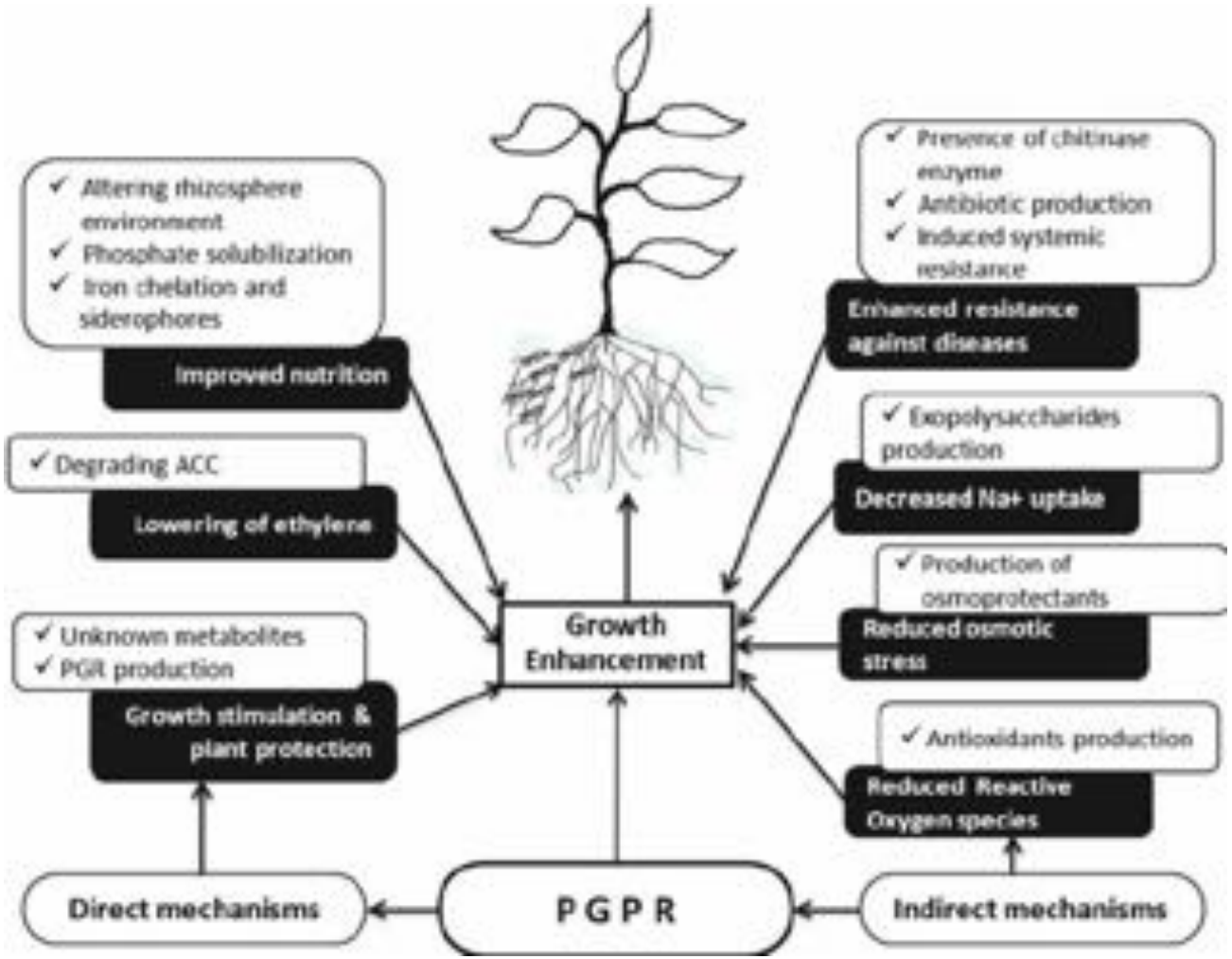
BIOSTIMULATION AND PGPR



Nadeem et al. (2013) Plant-microbe interactions for sustainable agriculture. DOI: 10.1007/978-81-322-1287-4_2

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OPEN QUESTIONS

- Soil microorganisms can indeed be a “holy grail” for remediation of environmental pollution. But:
  - How can we look for and find the best degraders?
  - How can we screen and apply them efficiently?
  - Can we couple degrading and plant growth promoting abilities?
  - Can we address very recalcitrant pollutants?
  - Is there still space for research?

Enrichment, selection and screening of degrading bacteria: results of the LIFE-BIOREST project

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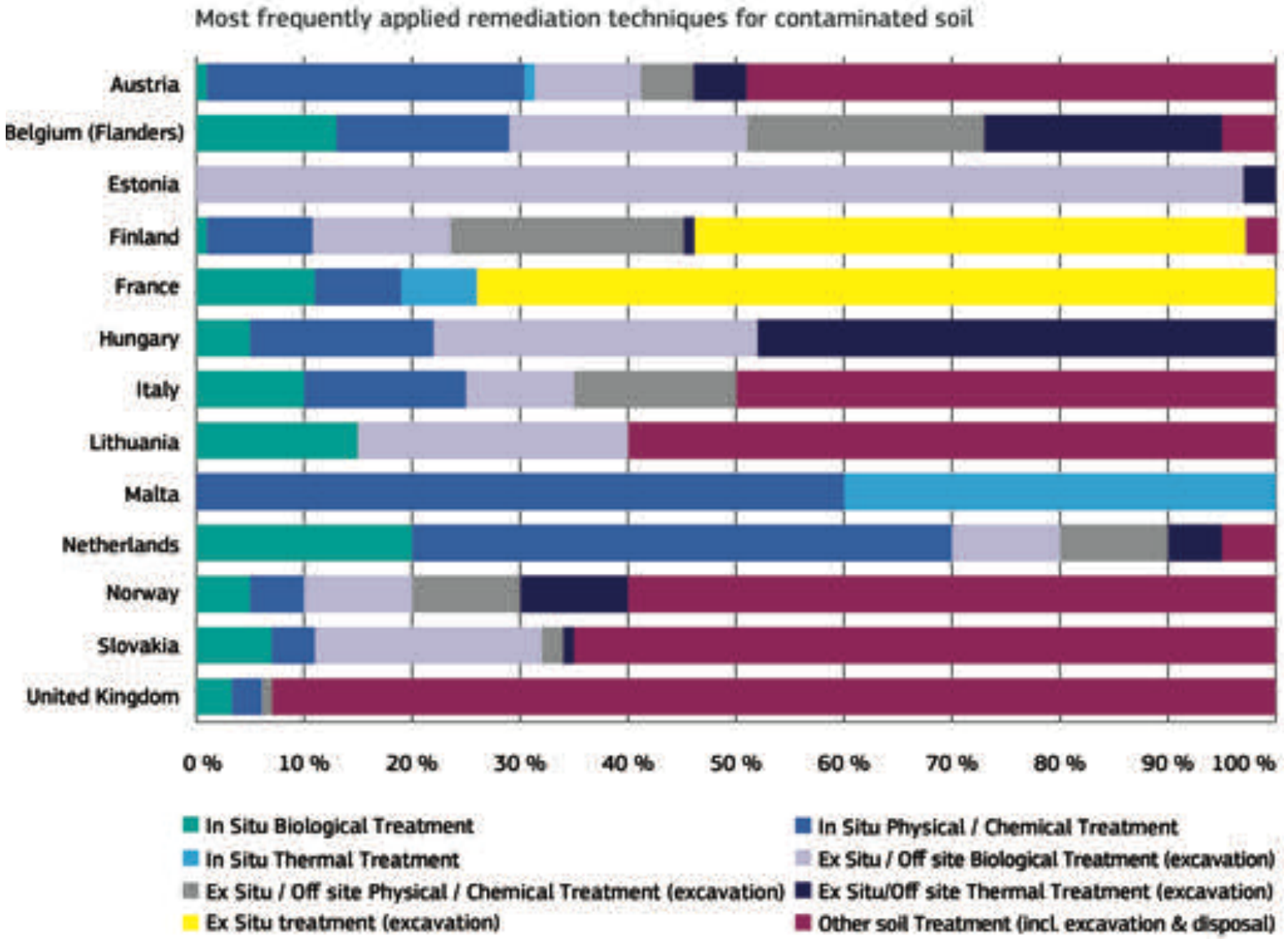
REMEDIATION IN THE EU



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LIFE-BIOREST OBJECTIVES

LIFE BIOREST proposes an biological method for *in loco* **remediation of hydrocarbons contaminated soils**.

1. Demonstrate the efficiency of a bioremediation approach for polluted soils based on the **BIOAUGMENTATION** with autochthonous, ecologically-adapted **BACTERIA** and **FUNGI**
2. Demonstrate the feasibility to **SCALE-UP** the production of microorganisms active in bioremediation.
3. Optimize protocols and **GUIDELINES FOR BIOREMEDIATION** that can be successfully applied in other scenarios.
4. Disseminate at the European level the clear societal benefits of addressing the **SOIL CONTAMINATION** issue.

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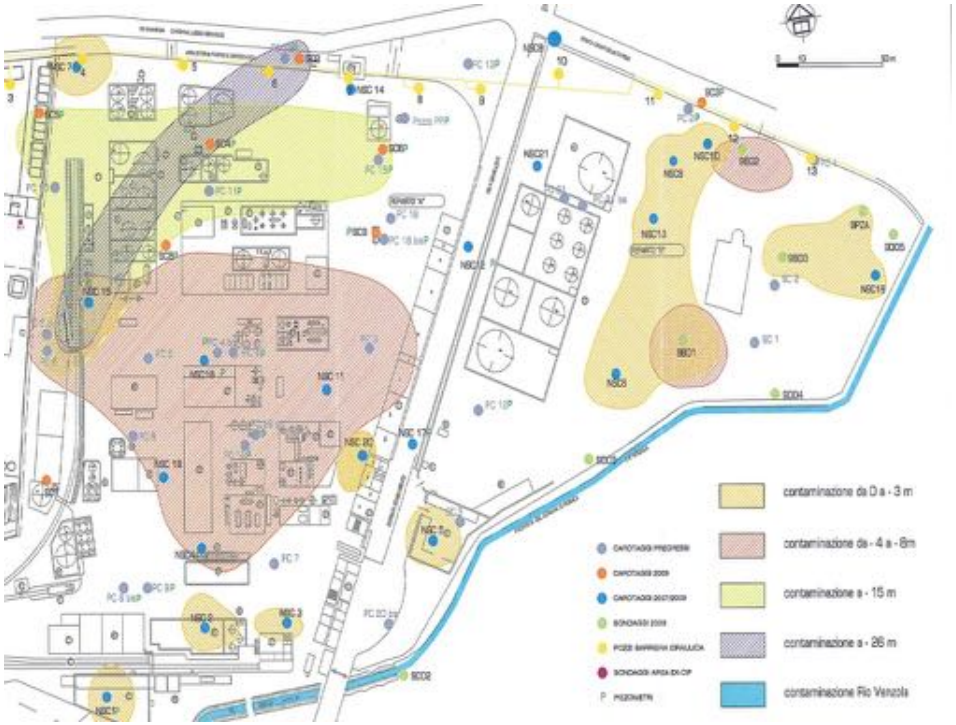
LIFE-BIOREST TEST SITE

Fidenza, Emilia Romagna

The experiments will be held at the Carbochimica industrial area, the national interest site (Sin) of Fidenza.



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LIFE-BIOREST ACTIVITIES

Implemetation action

ACTION B1 Optimized soil bioremediation by selected degrdating strains

ACTION B2 Upscaled production of microorganisms

ACTION B3 *In situ* bioremediation and revegetation

Monitoring action

Microbiological and ecotoxicological monitoring
LCA – Lyfe cycle assessment and socio-economical evaluation

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LIFE-BIOREST SCHEME AND NUMBERS

Polluted soil from Fidenza	4 different soils sampled, 3 different depths
↓	
Isolation and enrichment of degrading bacteria	192 strains
↓	
Dereplication and molecular identification of isolates	133 strains (20 pathogens)
↓	
Microtiter screening to quantify degrading efficiencies and select best isolates	113 strains X 6 pollutants = 798 measurements
↓	
Testing of best isolates and consortia on 100 g microcosms	35 microcosms X 2 replicates
↓	
Testing of best consortia on 15 kg mesocosms	6 mesocosms X 3 replicates
↓	
Testing of best consortium at biopile level	350 m^3 of soil treated

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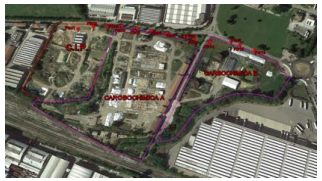
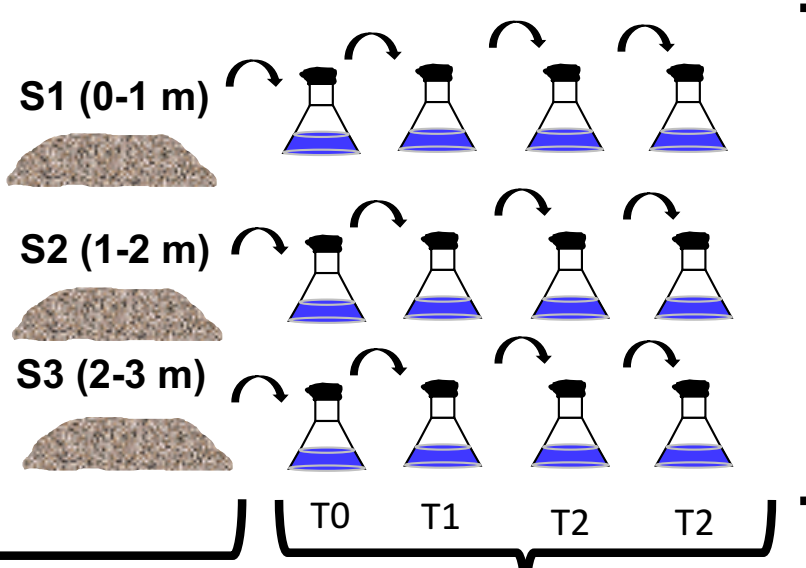
ENRICHMENTS - A MOLECULAR FOCUS





METHODS

Culturomics

- Plating of last enrichments
- Identification and screening of bacterial and fungal strains

Metagenomics

- DNA extraction
- PCR of 16S V3-V4 regions
- Illumina Miseq
- Bioinformatics

- Samples taken at 3 soil depths (1, 2 and 3 m)
- Sequential enrichments on M9+7 target pollutants as sole C source
- Samples for metagenomics taken from the original soil and every each enrichment step (1, 2 and 3 weeks)
- Strains isolation at the end of enrichment (3 weeks)

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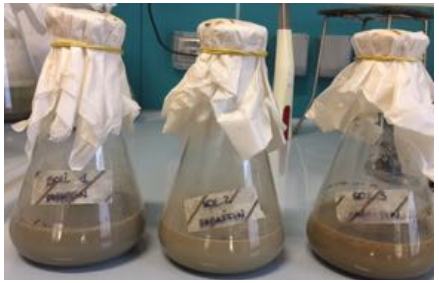
Enrichment Liquid Cultures

to favor development of microbial community capable of tolerating and degrading specific single contaminant

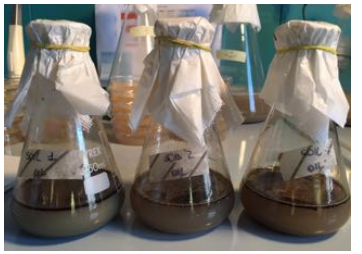
10g contaminated soil + MINERAL MEDIUM (M9) containing as sole carbon source

BENZENE

PARAFFIN OIL



CRUDE OIL



Three consecutive enrichment subcultures done in the same conditions

28 bacterial isolates

55 bacterial isolates

9 bacterial isolates

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ENRICHMENTS

BENZENE

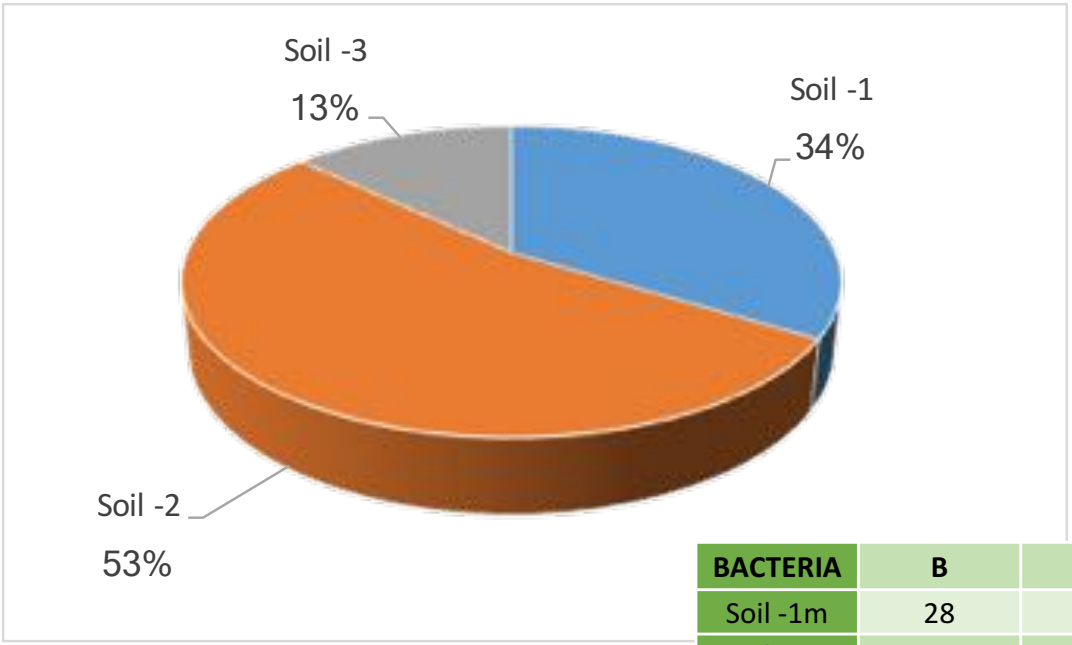
28 bacterial isolates

PARAFFIN OIL

55 bacterial isolates

CRUDE OIL

9 bacterial isolates



BACTERIA	B	N	P	Phe	Pa	O	total
Soil -1m	28	12	7	15	15	4	81
Soil -2m	18	14	6	12	34	9	93
Soil -3m	9	9	5	7	14	1	45
	25%	16%	10%	15%	29%	7%	**219**

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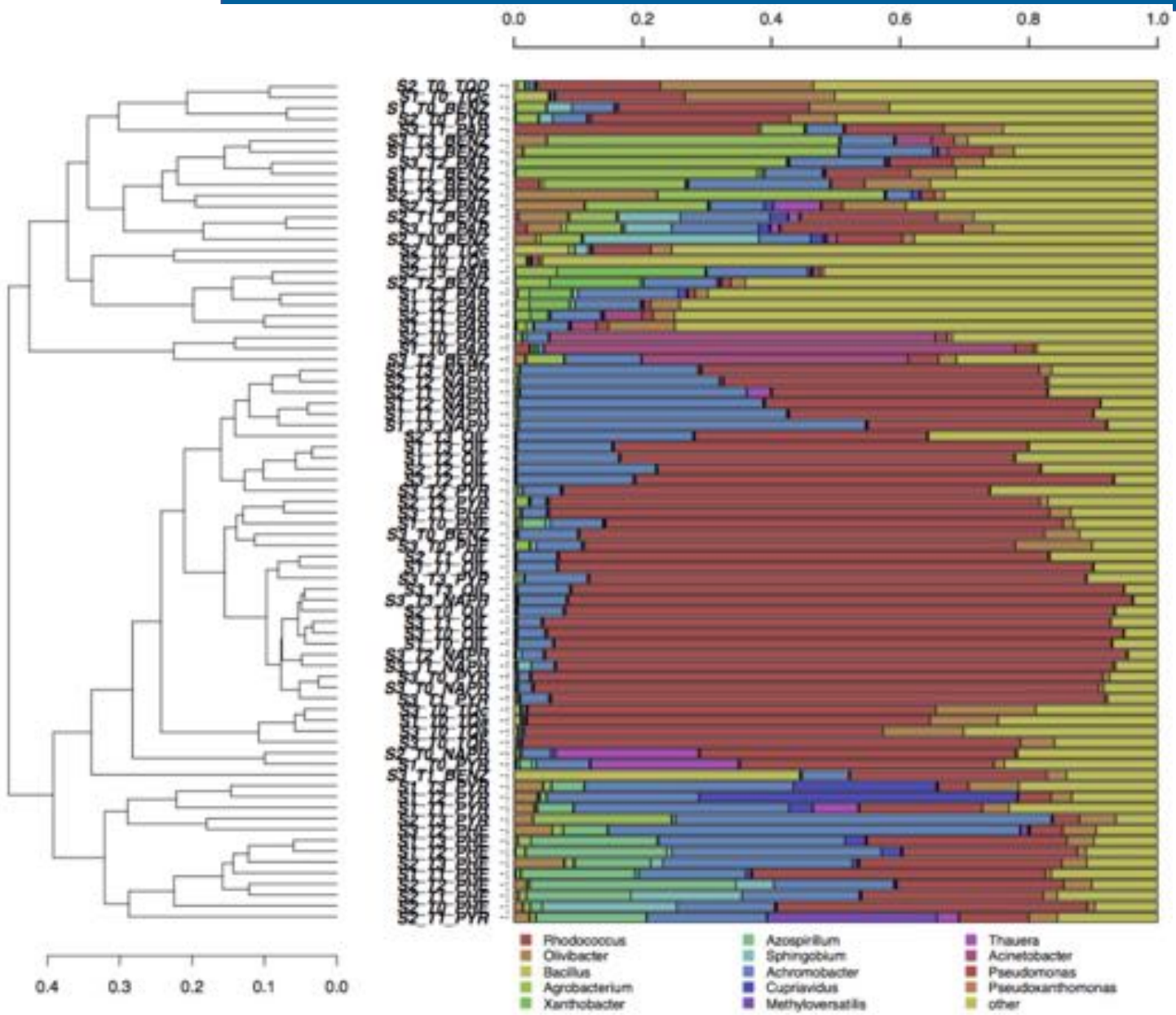
EVOLUTION OF BACTERIAL COMMUNITIES



Spini et al. **Molecular and microbiological insights on the enrichment procedures for the isolation of petroleum degrading strains…** *Frontiers in Mic* 2018

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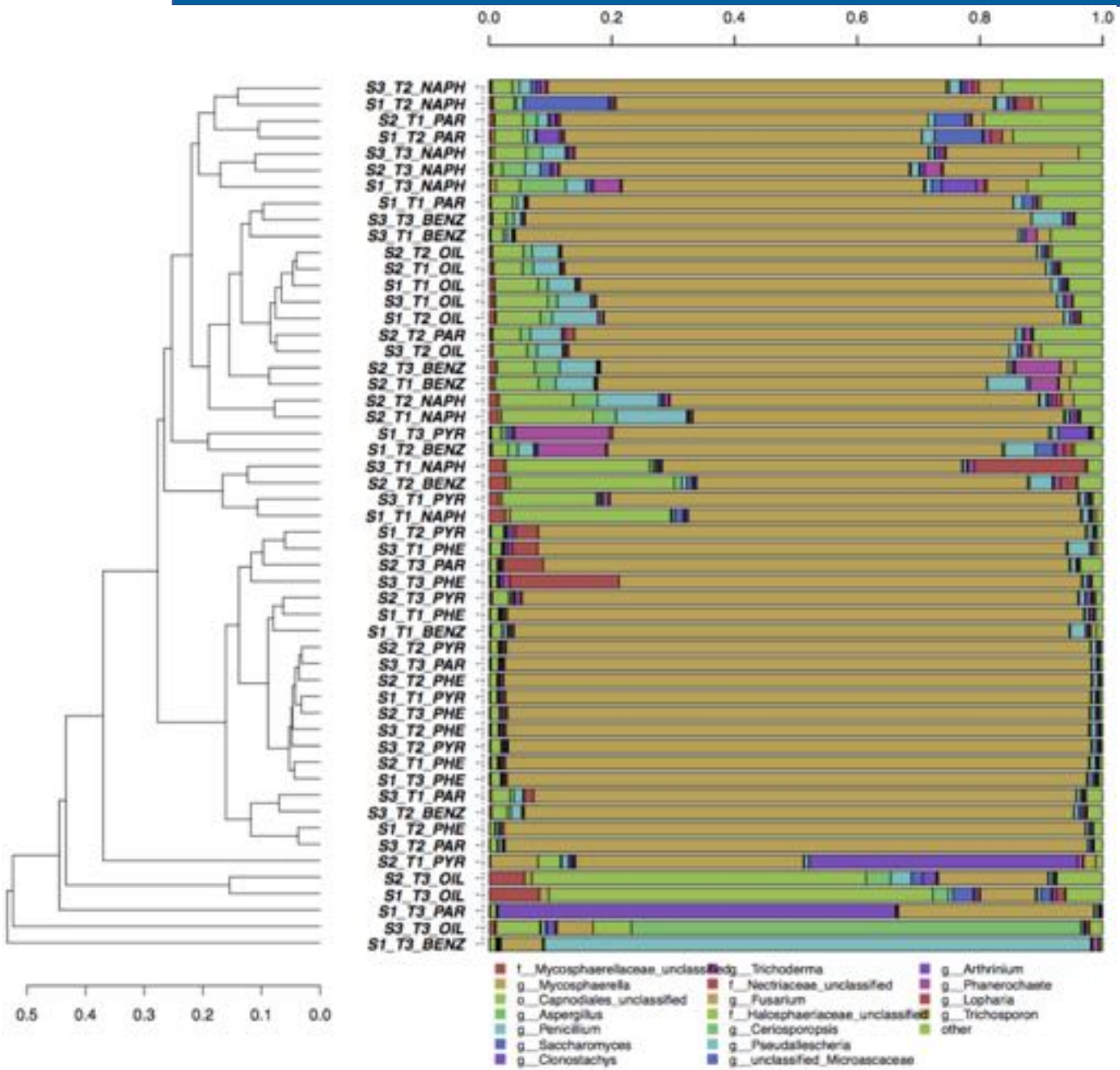
EVOLUTION OF FUNGAL COMMUNITIES



Spini et al. **Molecular and microbiological insights on the enrichment procedures for the isolation of petroleum degrading strains…** *Frontiers in Mic* 2018

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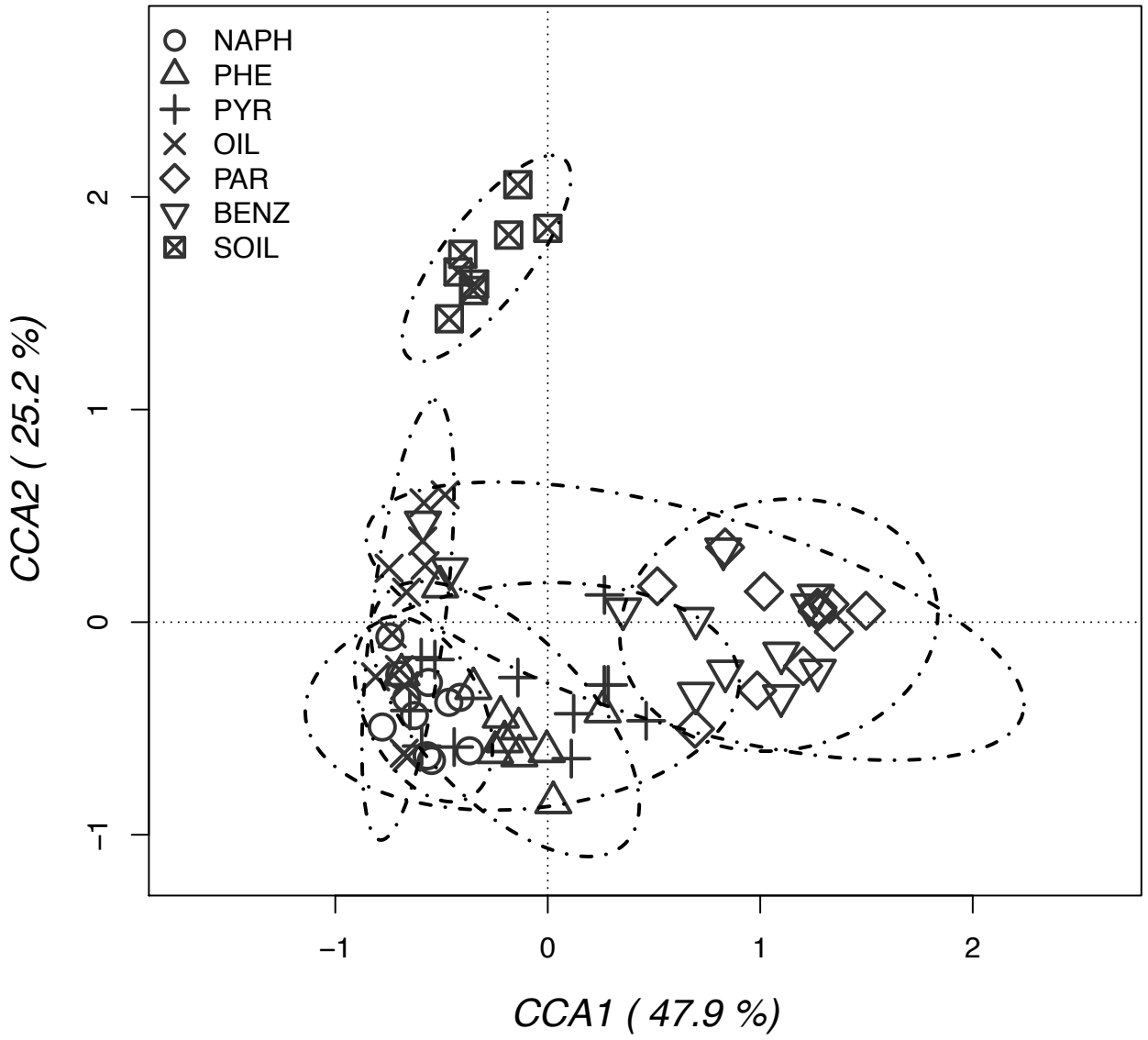
POLLUTANT EFFECT

Constrained Variance 36.1 %
model P= 0.001



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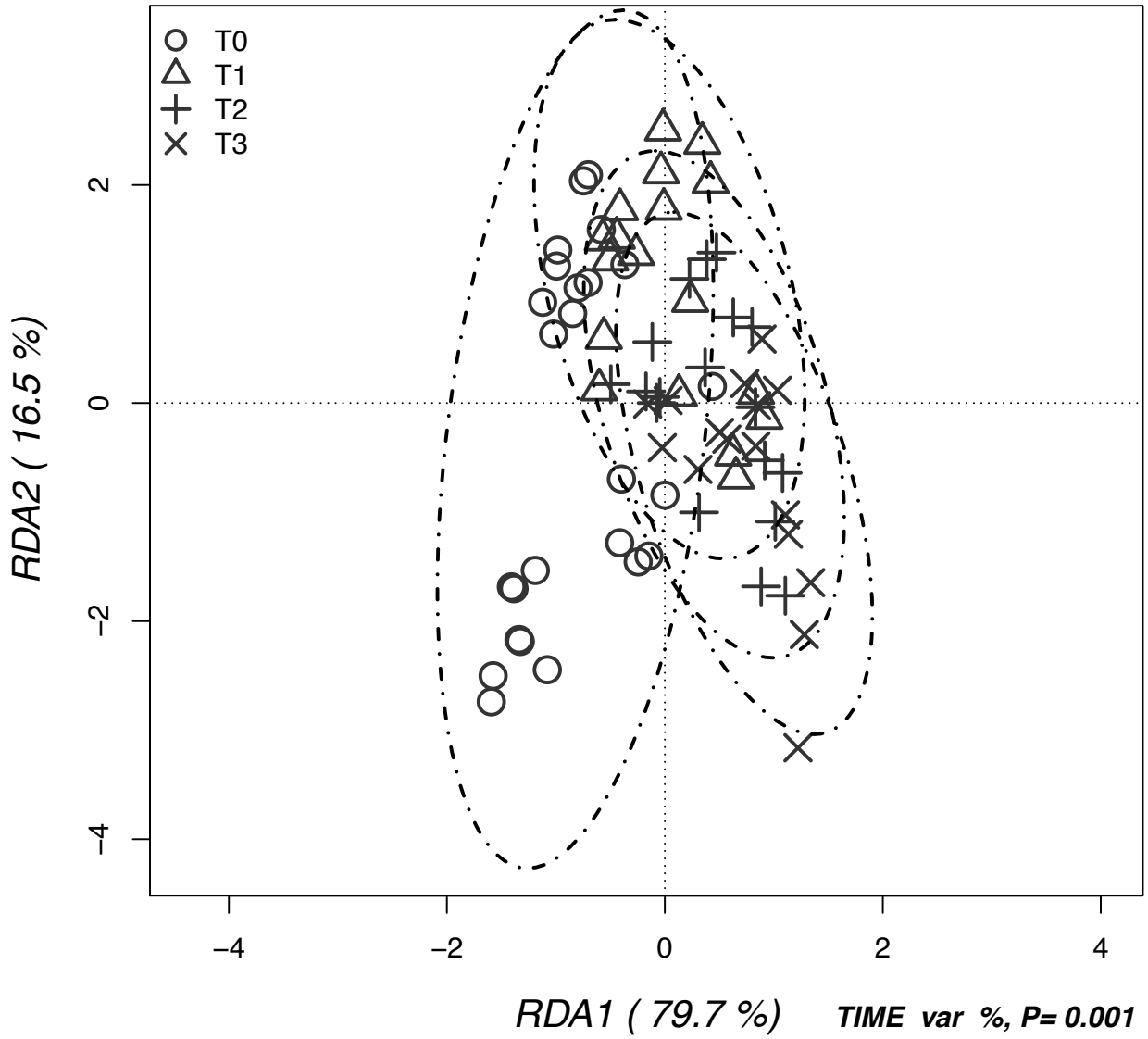
TIME EFFECT

Constrained Variance 8.3 %
model P= 0.002



TIME var %, P= 0.001

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SOIL DEPTH EFFECT

Constrained Variance 6.1 %
model P= 0.002

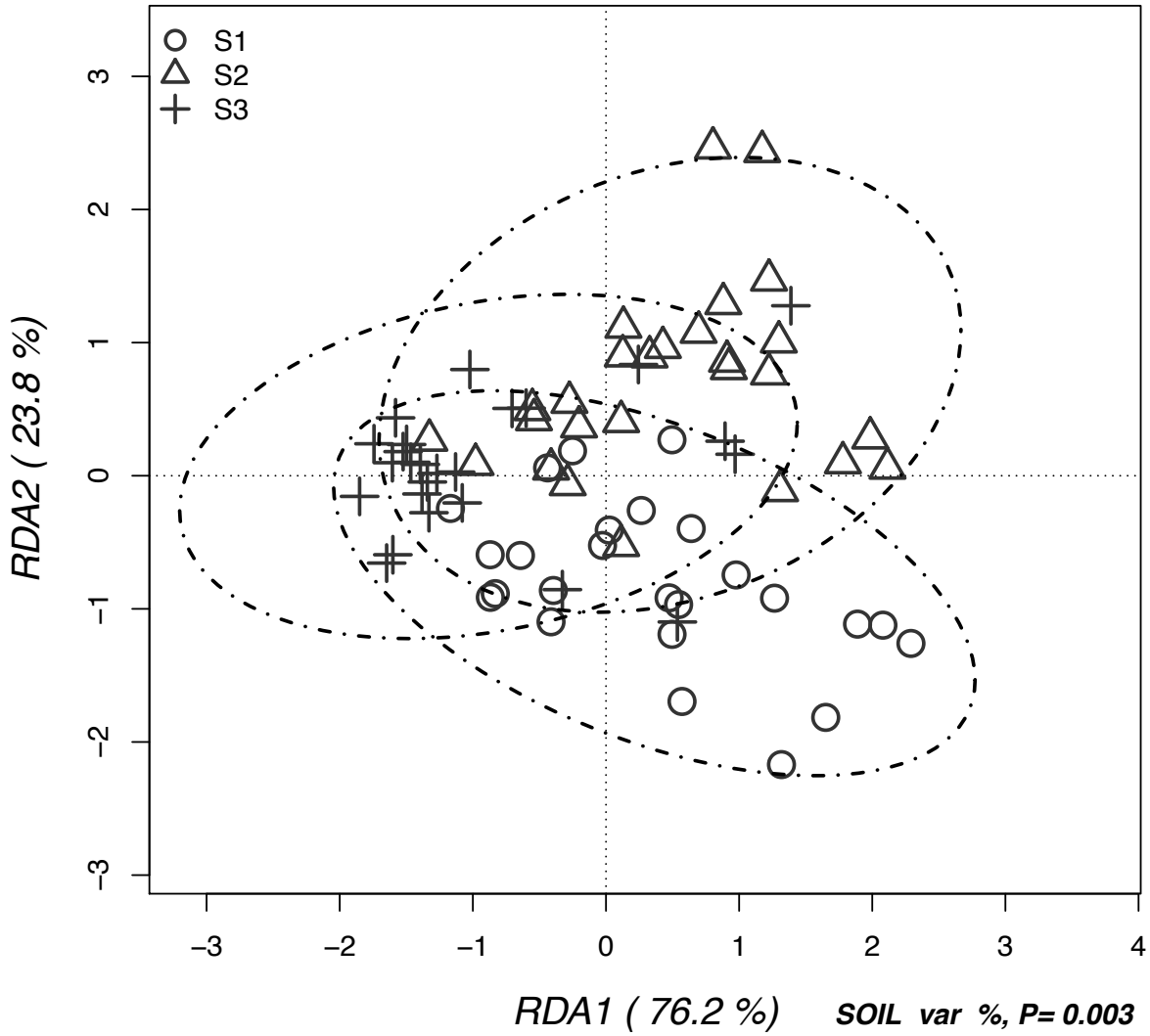

TABLE 2 | Relative abundances of bacterial genera determined by Illumina sequencing of 16S amplicns or by isolation on selective media; the comparison was performed at the end of the enrichment (step III).

Bacterial genus	16S Illumina			Bacterial isolates		
	S1	S2	S3	S1	S2	S3
Acholeplasma	nd	nd	nd	nd	nd	4,3
Achromobacter	26.68	29.37	8.39	7.7	4.4	4.3
Acinetobacter	0.38	0.18	1.60	nd	nd	4.3
Agrobacterium	9.08	11.79	11.62	nd	nd	nd
Ancylobacter	0.30	2.53	0.40	nd	nd	nd
Azospirillum	4.17	2.50	0.24	nd	nd	nd
Bacillus	0.11	0.09	0.09	7.7	8.9	nd
Cellulosimicrobium	0.03	0.02	0.08	nd	nd	4.3
Cupriavidus	4.64	0.46	0.08	nd	2.2	nd
Gordonia	3.00	0.04	0.06	3.8	nd	nd
Helicobacter	nd	nd	nd	nd	2.2	nd
Klebsiella	0.08	2.63	0.08	nd	nd	nd
Ochrobactrum	2.89	0.67	0.32	3.8	nd	nd
Olivibacter	1.13	6.57	1.28	nd	nd	nd
Paenibacillus	0.02	0.24	0.05	nd	2.2	nd
Pseudomonas	24.21	25.25	63.49	57.7	48.9	69.6
Pseudoxanthomonas	2.95	2.62	0.66	8.7	4.4	nd
Rhizobium	nd	nd	nd	nd	nd	4.3
Serratia	0.00	0.24	0.01	nd	6.7	nd
Shinella	2.79	0.25	0.03	nd	nd	nd
Sphingobacterium	0.95	0.94	1.60	3.8	17.8	nd
Stenotrophomonas	1.23	0.77	1.42	7.7	2.2	8.7

Data are expressed as percentages (among 10,065 sequences for Illumina data, among 96 strains for isolates).

TABLE 3 | Relative abundances of fungal genera as determined by Illumina sequencing of 16S PCR of amplicons or by isolation on selective media; the comparison was performed at the end of the enrichment (step III).

Fungal genus	ITS Illumina			Fungal isolates		
	S1	S2	S3	S1	S2	S3
Acremonium	0.001	0.003	nd	2.9	nd	nd
Arthrinium	2.0	0.4	0.3	nd	nd	nd
Aspergillus	1.8	2.0	0.8	8.6	8.7	19.4
Aureobasidium	0.9	0.3	0.3	2.9	nd	nd
Bjerkandera	nd	nd	nd	nd	4.3	nd
Capnodiales_unclassified	12.0	11.3	3.4	nd	nd	nd
Ceriosporopsis	0.2	0.2	14.8	nd	nd	nd
Cladosporium	0.2	0.6	0.1	8.6	13.0	nd
Clonostachys	11.0	0.6	0.5	2.9	nd	nd
Epicoccum	nd	nd	nd	2.9	4.3	
Eutypella	nd	nd	nd	nd	4.3	2.8
Fusarium	43.8	68.8	63.4	40.0	56.5	41.7
Hypocrea	nd	nd	nd	nd	nd	11.1
Irpex	nd	nd	nd	nd	4.3	nd
Penicillium	0.8	2.1	1.2	5.7	nd	2.8
Phanerochaete	0.5	1.7	0.3	nd	nd	nd
Polyporus	nd	nd	nd	nd	nd	2.8
Pseudallescheria	15.5	0.8	1.5	5.7	nd	nd
Scedosporium	0.01	0.01	0.12	nd	4.3	5.6
Sulcatispora	nd	nd	nd	nd	nd	2.8
Trametes	nd	0.1	0.04	nd	nd	2.8
Saccharomyces	0.9	0.7	0.1	nd	nd	nd
Trichoderma	3.6	0.4	0.5	20.0	nd	5.6
Wallemia	0.02	nd	0.02	nd	nd	2.8
Trichosporon	1.2	3.2	4.4	nd	nd	nd

Data are expressed as percentages (among 10065 sequences for Illumina data, among 94 strains for isolates).

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Molecular characterization by RAPD (Random Amplification of Polimorphic DNA) to discard replicates of the same bacterial isolate

Solid Screening

Enrichment liquid cultures

TOTAL BACTERIAL ISOLATES

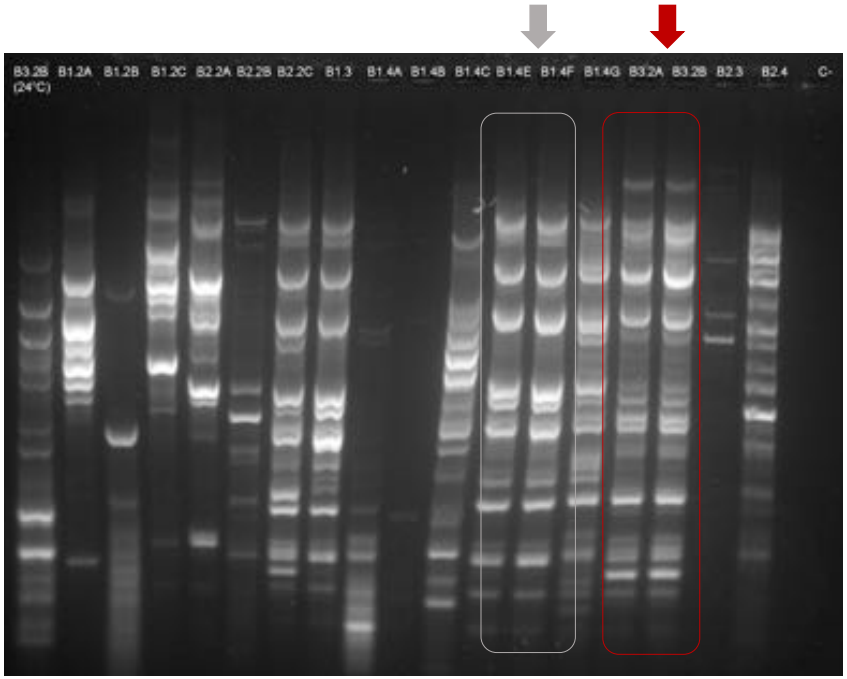


Figure. RAPD profiles of some bacteria isolated on paraffin, benzene and crude oil. Strains that are identical to each other give the same RAPD profile (highlighted in red and in green) and thus discarded to further steps.

68 UNIQUE ISOLATES from solid screening

65 UNIQUE ISOLATES from liquid enrichment

Taxonomical Identification of the bacterial strains by 16S rDNA gene sequencing

- Single colony Dna extraction
- PCR amplification of 16s rDNA gene by P1-P6 primers

Sequencing of the PCR products

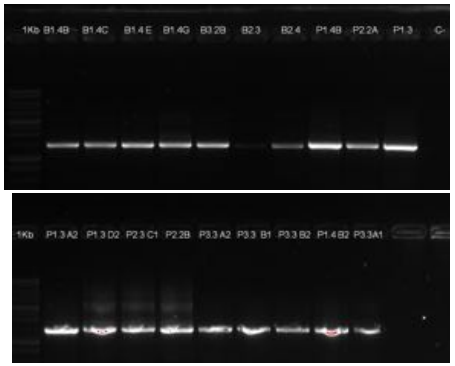
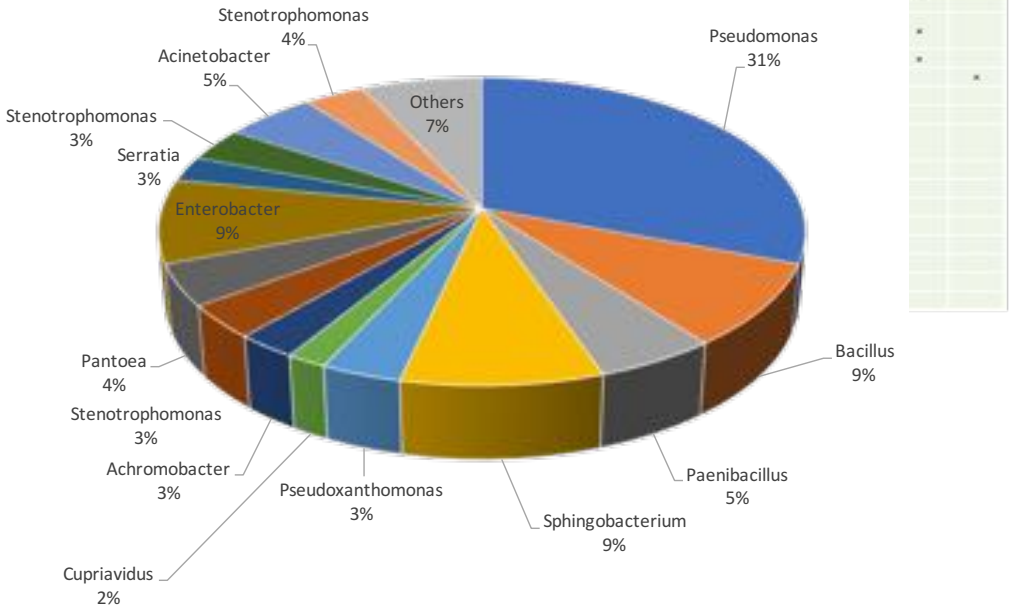


Figure. P1-P6 PCR products visualized on agarose gel (expected length 1500bp)

RDP Sequence match

Isolate number	RAPD best match-list of isolates	Solid Screening: Isolated from these cultural lines B	Pa	O	Solid Screening: Isolated from these soils s1	s2	s3	Liquid Enrichment: Isolated from these cultural lines B	Pa	O	Liquid Enrichment: Isolated from these soils s1	s2	s3
EC2	Helicobacter								x			x	
EC1	Gordonia rubripertincus								x		x		
EC9	Pseudomonas putida								x				x
EC8	Bacillus sp.								x		x		
EC5	Paenibacillus spp.								x			x	
EC18	Sphingobacterium multivorum								x			x	
EC17	Pseudomonas putida								x			x	
EC12	Pseudoxanthomonas spp.								x			x	
EC28	Pseudomonas putida								x				x
EC26	Achromobacter spp.								x		x		
EC22	Sphingobacterium multivorum								x		x		
EC30	Pseudomonas putida								x			x	
EC29	Pseudoxanthomonas mexicana								x		x		
EC34	Pseudoxanthomonas mexicana								x		x		
EC33	Achromobacter xylosoxidans								x			x	
CP1	Bacillus Subtilis								x		x		
EC62	Stenotrophomonas acidaminiphila								x			x	
CP14	Bacillus xiamenentis								x			x	
CP138	Sphingobacterium								x			x	
												x	
												x	
													x



Genera

- *Pseudomonas*
- *Bacillus/Paenibacillus*
- *Sphingobacterium*
- *Pseudoxanthomonas*
- *Enterobacter*
- *Acinetobacter*

n° **20** pathogen bacteria were discarded (according to the italian legislation, Dr.L 81/2008)

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96-wells Microplate Screening
to evaluate the degradative capacity of each isolate

6 tested substrates

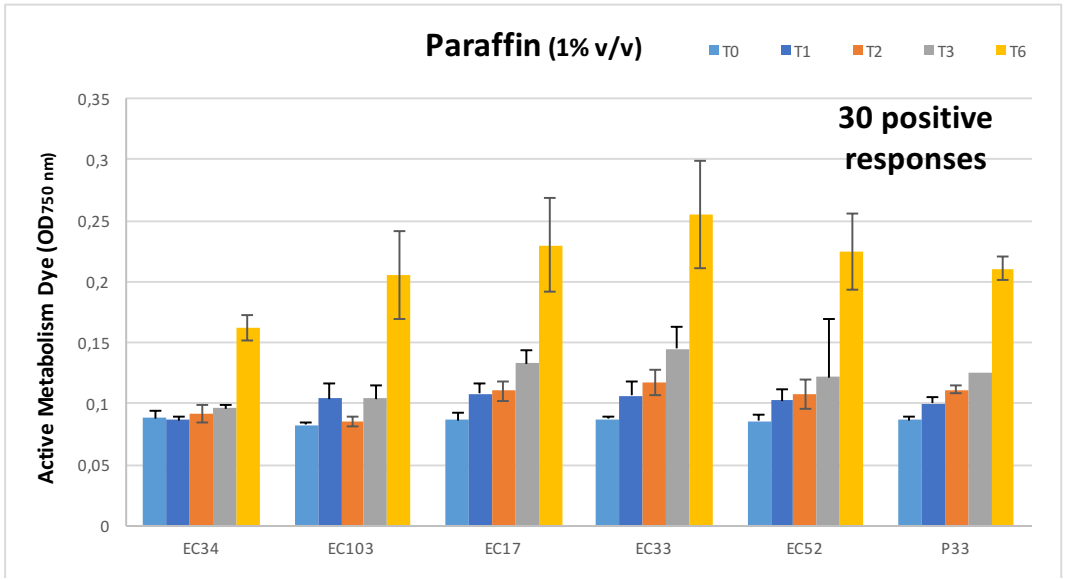
Pyrene
Phenanthrene
Heptadecane
Paraffin
Benzene
Naphtalene

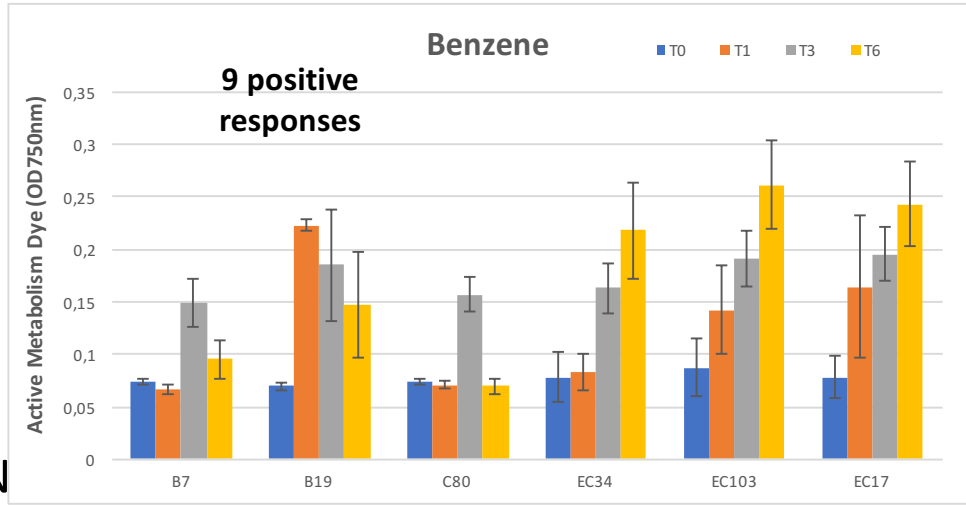
Each bacterial isolate

TO DATE

20 isolates are still under analysis

50 isolates are already analyzed





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96-wells Microplate Screening
to evaluate the degradative capacity of each isolate

Isolate ID	Genus/Species	Isolation approach	Isolation T (°C)	Substrate		Substrate		Substrate		Substrate	
EC1	Gordonia rubripertincus	enrichment culture	30°C	Naphtalene	(++) *	Paraffin	(++)				
EC33	Achromobacter xylosoxidans	enrichment culture	30°C	Heptadecane	(+)	Naphtalene	(++)	Benzene	(+++)	Paraffin	(++)
EC17	Pseudomonas putida	enrichment culture	30°C	Paraffin	(++)	Benzene	(+++)	Naphtalene	(++)		
EC34	Pseudoxanthomonas mexicana	enrichment culture	30°C	Naphtalene	(+++)	Benzene	(++)	Paraffin	(+)		
O7	Pseudomonas putida	solid screening	24°C	Paraffin	(+)						
O6	Pseudomonas putida	solid screening	24°C	Paraffin	(+)						
O2	Pseudomonas sp.	solid screening	24°C	Paraffin	(+)						
EC52	Sphingobacterium sp.	enrichment culture	30°C	Benzene	(+++)	Paraffin	(++)	Naphtalene	(++)		
B7	Bacillus idriensis	solid screening	24°C	Naphtalene	(++)	Benzene	(++)				
EC43	Pseudomonas fluorescens	enrichment culture	30°C	Paraffin	(+)						
B17	Pseudomonas chlororaphis	solid screening	24°C	Paraffin	(+)						
EC47	Pseudomonas fluorescens	enrichment culture	30°C	Paraffin	(++)						
EC103	Serratia marcescens	enrichment culture	30°C	Benzene	(+++)	Naphtalene	(+)	Paraffin	(++)		
EC80	Bacillus subtilis	enrichment culture	30°C	Benzene	(++)	Paraffin	(++)				
P31	Agrobacterium sp. SCAU685	solid screening	24°C	Paraffin	(+)						
P32b	Rhizobium sp.	solid screening	24°C	Paraffin	(++)						
P33	Rhizobium sp.	solid screening	24°C	Naphtalene	(++)	Benzene	(++)	Paraffin	(++)		
P37	biocide-degrading bacterium	solid screening	24°C	Paraffin	(+)						
B19	denitrifying bacterium SN230	solid screening	24°C	Benzene	(++)						
B20	Ochrobactrum sp.	solid screening	24°C	Paraffin	(+)						
P36	Agrobacterium sp.	solid screening	24°C	Paraffin	(+)						
EC42	Serratia marcescens	enrichment culture	30°C	Paraffin	(+)						
P34	Pseudomonas plecoglossicida	solid screening	24°C	Paraffin	(+)						
B11	Pseudomonas putida	solid screening	24°C	Pyrene	(+)	Phenanthrene	(++)	Paraffin	(++)		
O5	Pantoea agglomerans	solid screening	24°C	Paraffin	(++)						
P26	Pseudomonas putida	solid screening	24°C	Paraffin	(+)						
B10	Pseudomonas putida	solid screening	24°C	Paraffin	(++)						
P34B	Pseudomonas putida	solid screening	24°C	Benzene	(++)	Paraffin	**	Phenanthrene	(++)		
P27	Pseudomonas putida	solid screening	24°C	Paraffin	(++++)	Phenanthrene	(+)				
P30	Pseudomonas chlororaphis	solid screening	24°C	Paraffin	(++++)						
P28	Pseudomonas putida	solid screening	24°C	Paraffin	(+++)						
P29	Acinetobacter calcoaceticus	solid screening	24°C	Paraffin	(+++)						
B13	Pseudomonas putida	solid screening	24°C	Phenanthrene	(++)						

*percentage increase of the OD at 750nm after six days (t6) compared to the zero time (t0), normalized to the abiotic controls

(+) more than 150% (+++) greater than 300%

(++) between 200% and 300% (++++) greater than 400% (**) greater than 700%

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Isolate ID	Genus/Species	Isolation approach	Isolation T (°C)	OH isolation	Control	Naph	Benz	Pyr	Phe	Py	C17	Drop collaps.	OH displacem	Blue agar	pla throughf.	+ good ++ Very good, n. positive in all the tests summ		
OP7	Pseudomonas spp.	enrichment culture	30°C		100	1586	1784	0	0	467	846	+	+	-	+	50	4673	4723
R11 B1 3 24 A	Pseudomonas putida	solid screening	24°C		100	0	51	8569 (175%)	1293 (81%)	498	0	-	-	+	+	30	3193	3843
P27 P1.2.24 A	Pseudomonas sp.	solid screening	24°C		100	0	0	1348 (116%)	0	326	32	-	+++	++	++++	300	2932	3132
EC77	Acinetobacter calcoaceticus	enrichment culture	30°C		100	54	25	389 (35%)	334 (17%)	697	179	-	+++	-	++	100	1446	1546
R19 B2.2 24B	Rhizobium radiobacter	solid screening	24°C		100	13	447	95A (7%)	0	28	0	-	-	+	+	50	1442	1492
R7 B1.4.24E	Bacillus idriensis	solid screening	24°C		100	683	553	0	0	0	0	-	-	+	+	50	1203	1083
OG	Pseudomonas plecoglossicida	solid screening	30°C	x	100	0	25	851 (61%)	66	7	0	-	++	-	++	100	949	1049
R10 B3 3 24 A	Pseudomonas putida	solid screening	24°C		100	0	0	313 (23%)	0	199	114	-	-	-	-	0	991	991
R20 B2.2.24C	Ochrobactrum sp.	solid screening	24°C		100	0	0	605 (46%)	34	143	0	-	+	+	+	200	782	982
P27 P3 4 24 A	Pseudomonas putida	solid screening	24°C		100	0	0	130	0	169	79	-	-	-	+	50	314	964
P35 P1 3 24C	Pseudomonas putida	solid screening	24°C		100	0	0	458 (35%)	197	240	22	-	-	+	+	50	809	959
CP122	Sphingobacterium sp.	enrichment culture	30°C		100	134	86	61	121	229	113	-	+	-	+	50	750	800
OS	Pseudomonas putida	solid screening	30°C	x	100	0	0	661 (51%)	57	162	0	-	++	+	++	100	690	790
R23 B1.3 24A	Acinetobacter spp.	solid screening	24°C		100	86	84	101	104	127	141	-	-	++	++	150	837	737
EC101	Serratia marcescens	enrichment culture	30°C		100	102	70	0	244	286	0	-	+	-	+	50	683	733
P36.24 P1.3 24A	Agrobacterium sp.	solid screening	24°C		100	11	5	0	215	353	0	-	+	+	++	100	623	723
OD	Pseudomonas sp.	solid screening	30°C	x	100	0	0	41A (3%)	12	91	0	+	+++	+	++++	200	518	718
P29 P2 3 24 B	Acinetobacter calcoaceticus	solid screening	24°C		100	0	0	0	58	656	0	-	-	-	-	0	714	714
R15 B2 2 37B	Rhizobiaceae bacterium	solid screening	37°C		100	35	140	58	0	309	0	+	+++	-	++	100	542	642
OJ7	Pseudomonas putida	solid screening	30°C	x	100	0	16	102 (10)	91	119	94	-	-	+	+	50	591	641
EC91	Cupriavidus sp.	enrichment culture	30°C		100	12	97	139	125	142	125	-	+	-	+	50	580	630
EC30	Pseudomonas putida	enrichment culture	30°C		100	0	0	0	0	478	139	-	-	-	-	0	611	611
P34B P1 3 24 C	Pseudomonas putida	solid screening	24°C		100	25	84	79	94	279	7	-	-	-	+	50	562	612
R13 B1 3 24 C	Pseudomonas putida	solid screening	24°C		100	0	0	199	279	85	0	-	-	-	+	50	562	612
R14 B2 2 37A	Agrobacterium sp.	solid screening	37°C		100	109	0	232	118	98	0	-	+	+	+	50	557	607
EC26	Achromobacter spp.	enrichment culture	30°C		100	0	50	0	33	408	0	-	++	-	++	100	590	590
CP16	Acholeplasma vituli	enrichment culture	30°C		100	75	85	76	142	134	94	-	-	-	-	0	581	581
CP23	Pseudomonas putida	enrichment culture	30°C		100	33	89	0	289	184	0	-	-	+	+	50	525	575
R23 B2.2 4A	Acinetobacter spp.	solid screening	24°C		100	17	50	72	81	205	68	-	+	-	++	100	473	573
R9 B1.4.24G	Bacillus simplex	solid screening	24°C		100	82	101	92	97	94	57	+	+	-	+	50	523	573
R14 B2 2 37A	Rhizobiaceae bacterium	solid screening	37°C		100	0	0	80	0	488	0	-	+	-	+	50	516	566
B4 B1.4.24 C	Bacillus idriensis	solid screening	24°C		100	12	44	78	129	224	74	-	-	-	-	0	559	559
P32b P1.4 24A	Rhizobium sp.	solid screening	24°C		100	0	0	214	20	126	0	+	+	++	+	200	359	559
EC43	Pseudomonas fluorescens	enrichment culture	30°C	x	100	0	0	0	180	278	0	-	+	+	+++	300	459	359
R13	Pseudomonas putida	solid screening	24°C		100	0	93	100	94	308	0	-	-	-	-	0	554	554
EC103	Serratia marcescens	enrichment culture	30°C		100	90	135	159	0	15	46	-	+	+	++	100	442	542
EC28	Sphingobacterium multivorum	enrichment culture	30°C		100	121	102	94	88	78	96	-	-	-	-	0	530	530
CP188	Sphingobacterium multivorum	enrichment culture	30°C		100	0	51	4	0	246	171	-	+	-	+	50	474	524
R13 B1 2 24C	Pseudomonas putida	solid screening	24°C		100	102	88	0	284	44	0	-	-	-	-	0	518	518
P34 p1.3 24G	Pseudomonas plecoglossicida	solid screening	24°C		100	0	4	0	127	173	0	-	+++	-	++++	300	300	300
EC2	Helicobacter	enrichment culture	30°C		100	12	5	191	106	362	0	-	-	-	-	0	323	323
R1 B B1 4 24G	Bacillus simplex	solid screening	24°C		100	26	82	52	19	186	57	-	+	-	+	50	412	462
EC5	Paenibacillus spp.	enrichment culture	30°C		100	0	48	48	19	199	200	-	-	-	-	0	416	416
EC33	Achromobacter xylosoxidans	enrichment culture	30°C		100	83	141	90	7	89	18	-	-	-	-	0	438	438
EC42	Serratia marcescens	enrichment culture	30°C	x	100	0	74	163	0	0	0	+	+++	+	++++	200	237	437
EC52	Sphingobacterium sp.	enrichment culture	30°C		100	101	148	134	0	95	11	-	-	-	-	0	435	435
EC60	Pseudomonas mosselii	enrichment culture	30°C		100	26	80	0	0	207	110	-	-	-	-	0	413	413
EC74	Stenotrophomonas maltophilia	enrichment culture	30°C		100	11	0	84	35	213	21	-	+	+	+	50	372	422
EC17	Pseudomonas putida	enrichment culture	30°C		100	91	129	106	8	80	16	-	-	-	-	0	415	415
R12 B1.2.37F	Bacillus sp.	solid screening	37°C		100	33	46	80	83	113	84	-	-	-	-	0	416	416
B26 B1.4.24B	Georgenia daeguensis	solid screening	24°C		100	12	26	93	104	142	57	-	-	-	-	0	406	406
CP4	Cupriavidus campinensis	enrichment culture	30°C		100	5	12	79	101	110	96	-	-	-	-	0	404	404
P34-24 P1.2 24G	Pseudomonas sp.	enrichment culture	24°C		100	0	4	0	127	173	0	-	+++	+	++	100	303	403

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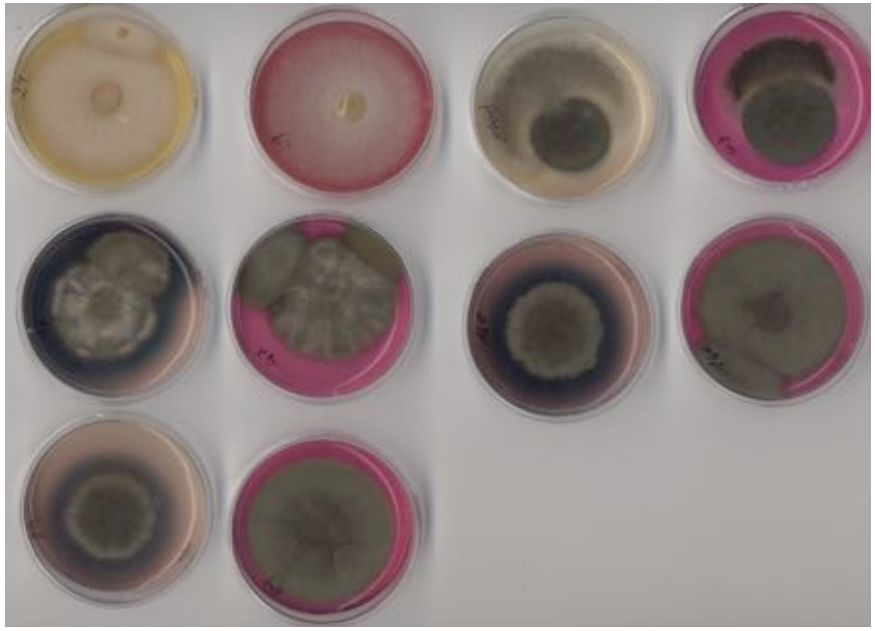
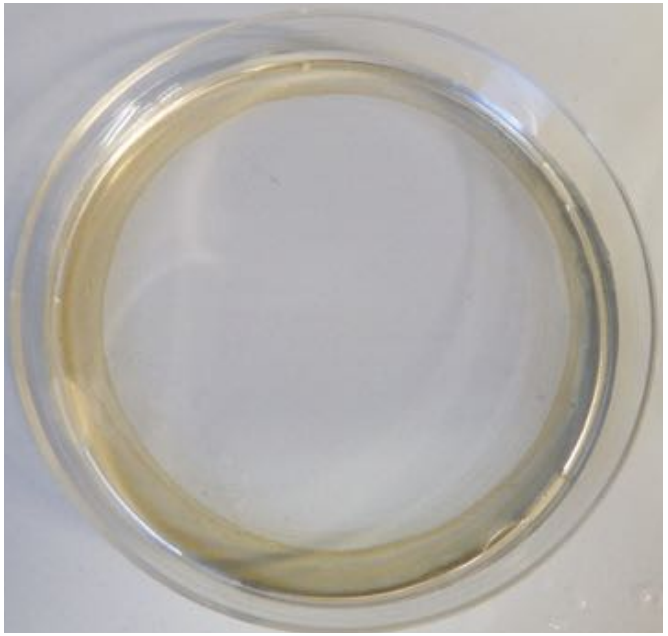
Screening of isolates capable of producing biosurfactants or redox enzymes:

- Colorimetric tests using redox dyes (ABTS, Poly R478)
- Selection of the best tests for biosurfactant productions (Oil spread test, emulsification test, etc.)





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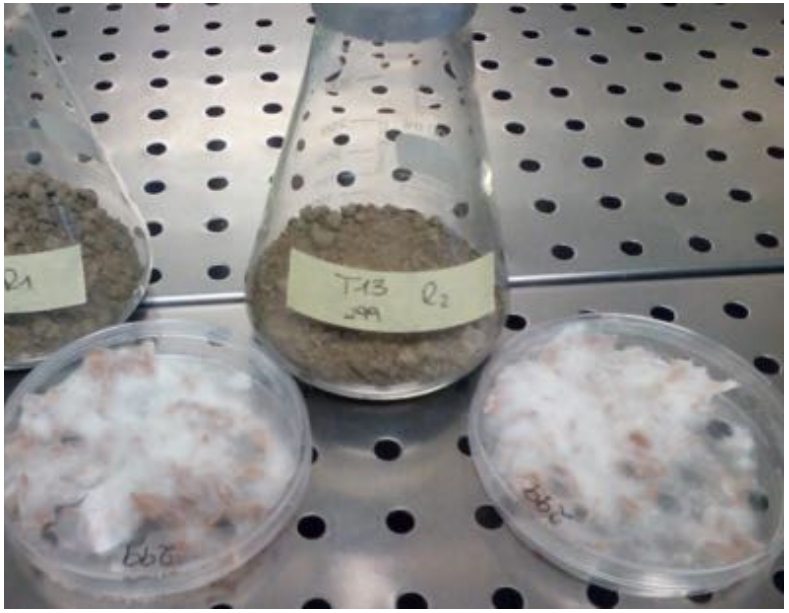
MICROCOSMS SELECTION



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MICROCOSMS SELECTION

	Micro cosm/ thesis	strains	Rationale
bacteria	1	CP7	1st best degrader
	2	B11	2nd best degrader
	3	EC77	3rd best degrader
	4	CP7+B11+EC77	first best 3
	5	CP7+B11+EC77+P34-24	first best 3 + biosurfactant prouducer
	6	O3	good pyr degrader (isolated from oil)
	7	O3+P34-24	good pyr degrader + biosurf (bioavailability issue)
	8	O6	degrader isolated from oil
	9	EC31	versatile on different pollutants
	10	CP122	versatile on different pollutants
fungi	11	127	first best fungus
	12	71	second best fungus
	13	299	third best fungus
	14	127 + 71 + 299	3 best fungi
	15	219 + 15 + 304 + 117	best mitosporic fungi
	16	219 + 15 + 304 + 117 + 127	best mitosporic fungi + basidiomycete
	17	219 + 15 + 304 + 117 + 127 + 71 + 299	best mitosporic fungi + 3 best fungi
	18	51 + 203	among the best tens, different growth, biosurfactant producers
	19	304 + 117 + 307 + 308	isolated from oil
	20	304 + 117 + 307 + 308 + 127	isolated from oil + basidiomycete

	Microcosm/thesis	strains	Rationale
fungi+bacteria	21	127 + 71 + 299 + EC77 + B11 + CP7 + +P37	3 best fungi and bacteria
	22	219 + 15 + 304 + 117 + 127 +299 + EC77 + B11 + CP7 + P37	T17 + 3 best bacteria
	23	304 + 117 + O3 + O6	isolated from oil
	24	304 + 117 + O3 + O6 + 127 + 299	isolated from oil + basidiomycete
	25	304 + 51 + 299 + CP7 + EC77 + P37	those that seem to grow on soil + basidiomycete + best bacteria
	26	219+15+304+117+148+ 06+EC31+CP122+ CP7+EC77	best fungi and bacteria accoridng to 20d data of microcosms
	27	304+117+307+308+299+148 + 06+EC31+CP122+EC77+B11+P37	best fungi and bacteria according to 20d data of microcosms+ best biosurfactant bacterial producers
	28	143 + 131 + 307 + 188 + 177 + 06+EC31+CP122+P29+EC30+EC101	fungi with high growth in microplate + best bacteria in microcosm+ alkanes bacterial degraders
	29	245 + 239 + 203+307 + 131 + 188 + 299 + B24+ EC101+EC77+P36+EC31	fungi isolated in enrich + pyr/phe growing fungi + versatile bacteria on different pollutants
	30	203 + 274 + 177+51+148 + 299 + P29+EC30+03+B19+B20+B10	pyr or phe or alkanes growing fungi + basidiomycete + pyr/phe and alkanes bacteria degraders
fungi+bacteria +biosurfactant	31	Thesis 21 + biosurfactants	Thesis 21 + biosurfactant (bioavailability issue)
	32	Thesis 22 + biosurfactants	Thesis 22 + biosurfactant (bioavailability issue)
	33	Thesis 26+ biosurfactants	Thesis 26+ biosurfactant (bioavailability issue)
	34	Thesis 27+ biosurfactants	Thesis 27+ biosurfactants (bioavailability issue)
	35	Thesis 29+ biosurfactants	Thesis 29+ biosurfactants (bioavailability issue)

theses	2ringsPAHs	3ringsPAHs	4,5,6PAHs	C>12	final_score
T1	0	1	1	0	2
T2	0	1	1	0	2
T3	0	1	1	0	2
T4	0	1	1	0	2
T4R	1	1	0	1	3
T5	1	1	0	0	2
T6	1	1	1	0	3
T7	0	1	0	0	1
T8	0	0	0	0	0
T9	0	1	1	0	2
T10	0	1	1	0	2
T11	1	1	1	0	3
T12	0	1	1	0	2
T13	0	1	0	0	1
T14	1	1	1	0	3
T15	0	1	0	0	1
T16	0	1	1	0	2
T17	1	0	0	0	1
T18	1	1	0	0	2
T19	1	0	0	0	1
T20	1	0	0	0	1
T21	0	1	0	0	1
T22	0	1	1	1	3
T23	1	1	0	0	2
T24	0	1	0	1	2
T25	0	1	1	1	3
T26	1	1	0	1	3
T27	1	1	0	0	2
T28	1	1	0	0	2
T29	1	1	0	0	2
T30	1	1	0	0	2
T31	1	0	0	0	1
T32	0	0	0	0	0
T33	1	1	0	0	2
T34	1	1	0	0	2
T35	1	1	0	0	2

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MESOCOSMS

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MESOCOSMS SELECTION

Mesocosms theses	Corresponding microcosms	Composition
A	22	219 + 15 + 304 + 117 + 127 +299 + EC77 + B11 + CP7 + P37
B	32	219 + 15 + 304 + 117 + 127 +299 + EC77 + B11 + CP7 + P37
C	27	304+117+307+308+299+148 + 06+EC31+CP122+EC77+B11+P37
D	28+biosurfact	143 + 131 + 307 + 188 + 177 + 06+EC31+CP122+P29+EC30+EC101
E	29	245 + 239 + 203+307 + 131 + 188 + 299 + B24+ EC101+EC77+P36+EC31
F	35	203 + 274 + 177+51+148 + 299 + P29+EC30+03+B19+B20+B10

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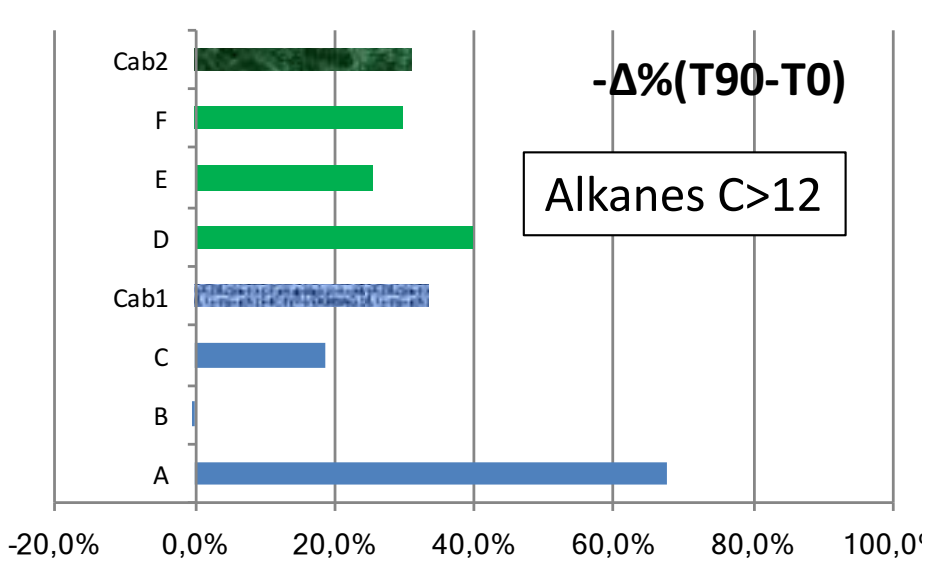
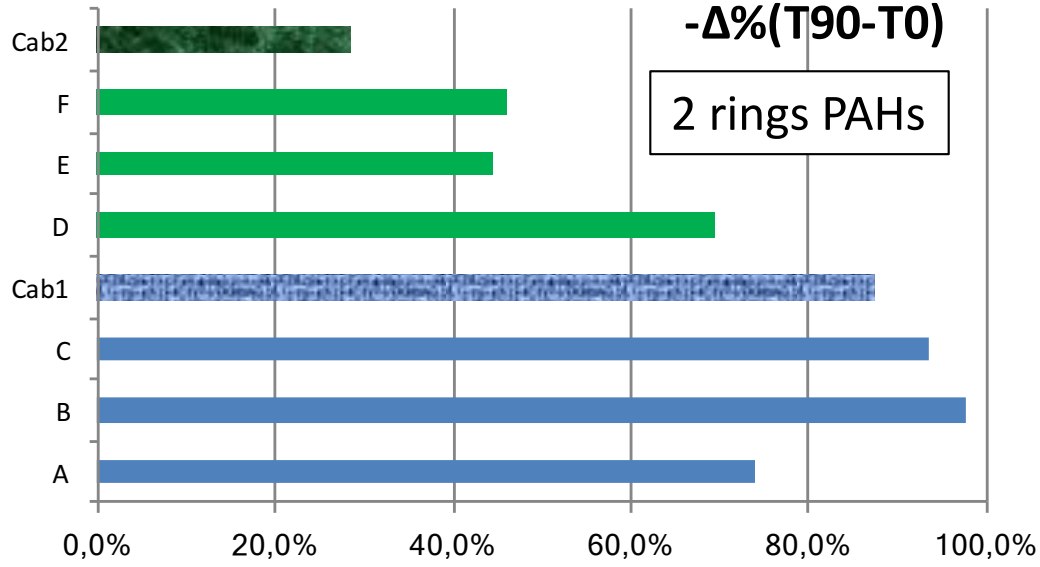
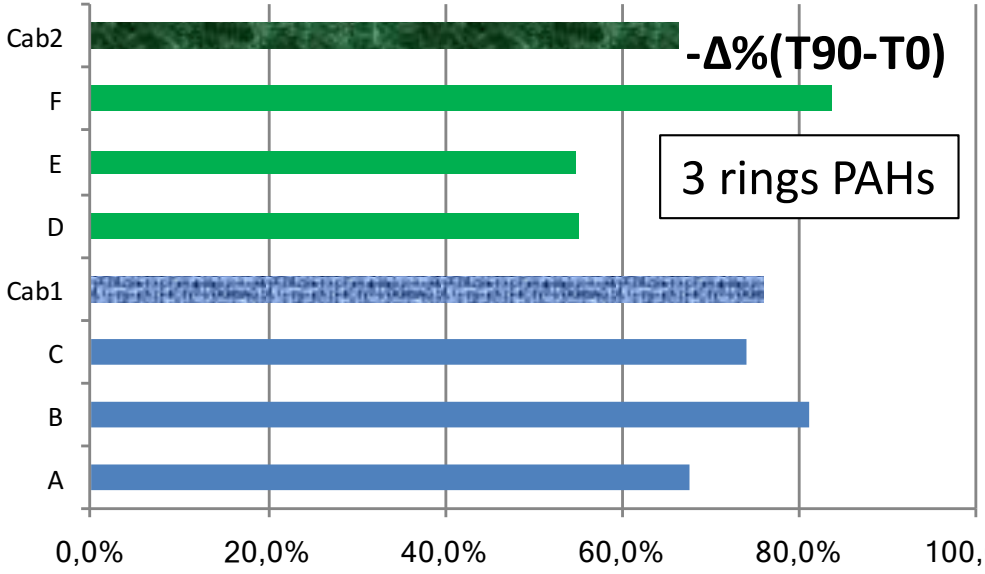
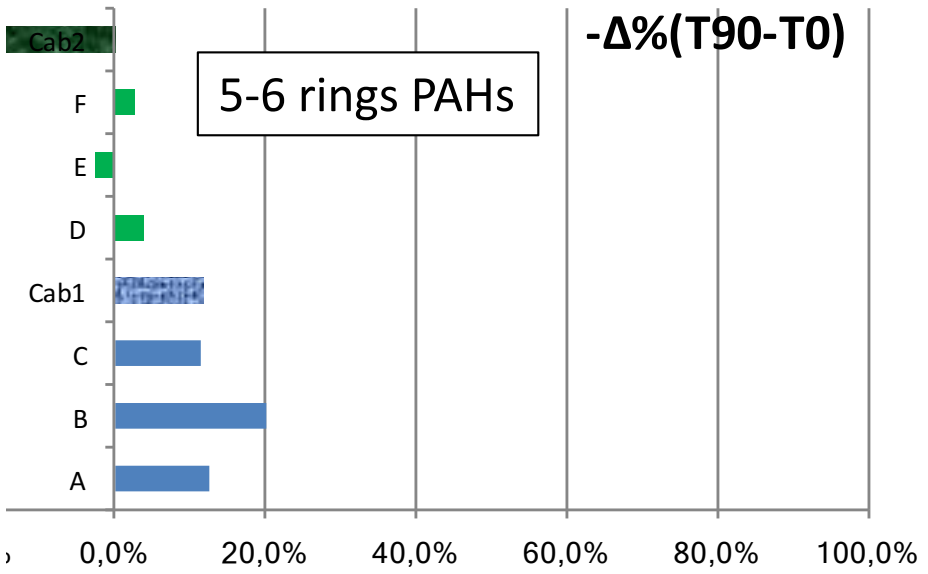
MESOCOSMS RESULTS









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CONCLUSIONS

- Soils are immense reservoirs of microbial diversity, still mostly unexplored
- Culturomics and molecular methods can be efficiently coupled to exploit this diversity
- Final goal is to cultivate and produce the best strains: screening methods are fundamental
- Recalcitrant molecules can be addressed (
- Promising results can be obtained by coupling PGPR and degrading abilities, as well as different organisms (bacteria, fungi and plants)

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