Polycyclic aromatic hydrocarbons (PAHs) bioremediation of a contaminated soil
Isolation of native bacterial strains

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INTRODUCTION

20th century was the golden age of steel industry. The consequence of this past is nowadays the presence of **multiple wastelands severely polluted mainly by PAHs (Polycyclic Aromatic Hydrocarbons), BTEX (Benzene, Toluene, Ethylbenzene, Xylene) and heavy metals**. The reallocation of those sites for new activities is a major challenge and requires preliminary soil remediation to obtain pollution levels below the legislation limits. The traditional technique used for soil remediation in Wallonia (Belgium) includes the excavation of contaminated soils, transport and treatment *ex situ* in specialised centres. However, this procedure is very expensive, may present health risks for local population and leads to negative carbon footprint. Consequently, ***in situ* decontamination methods** must be considered.

In this context, the **MEMORIS project (Treatment MEthodology and MOnitoring for sequenced Reallocation of severely polluted Industrial Sites)** was initiated. Its main goal is the **combination of treatment and monitoring methods allowing the sequential reuse of a severely polluted industrial site in order to decrease the financial impact of sanitation costs**. For this purpose, different techniques will be combined, namely, **bioremediation** (microbial degradation of PAHs and BTEX), **phytoremediation** (phytostabilization of heavy metals), **thermal treatment of the soil** (stimulation of the microbial activity), **monitoring** (pollution evolution in continuous and during long periods) and **health risk assessment** (application of bio-indicators (invertebrates) and transposition of ecotoxicological tests to assess the impact of pollution on human health).

It is well-known that some micro-organisms such as **bacteria** and **fungi** are involved in the bioremediation of aromatic compounds. Those micro-organisms can either grow on a medium using PAHs as sole carbon and energy sources (mainly for bacteria) or they can produce **enzymes** such as **lignin peroxidase** (**LiP**), **manganese peroxidase** (**MnP**) or **laccase** (**LAC**) (mainly for fungi and, more exactly white rot fungi) which have degradative properties on aromatic compounds. Those enzymes can catalyse one-electron oxidation of PAHs with formation of PAH quinones [1,2,3].

ISOLATION OF NATIVE MICRO-ORGANISMS

- Location of polluted site: old coking plant, city of Charleroi (Belgium)
- Type of pollutants in the working area: PAHs, BTEX, heavy metals and mineral oils

Enrichment of soil and water samples

- Culture medium: BHM [4,5] or M9 [6] + soil or water sample + PAHs mixture (naphthalene, anthracene and pyrene)
- Incubation: 30°C, orbital shaking 130 rpm
- Subculturing: every 3 weeks
- Isolation on Petri dishes (MSM + cycloheximide (fungi) ; Sabouraud + chloramphenicol (bacteria) ; LB)

PCR amplification

- Bacterial set 8F/1492R: 30 s at 98°C, followed by 40 cycles of: 10 s at 98°C, 20 s at 55°C, 45 s at 72°C, and final extension for 5 min at 72°C
- Fungal set FR1/NS1: 30 s at 98°C, followed by 40 cycles of: 10 s at 98°C, 20 s at 47°C, 45 s at 72°C, and final extension for 5 min at 72°C
- Sequencing results are aligned using CodonCode Aligner and compared to the GenBank nucleotide database using the BLAST (NCBI)

Isolated strains

- *Achromobacter sp.*
- *Bacillus cereus*
- *Bacillus licheniformis*
- *Bacillus thurigiensis* (Fig. 1)
- *Bacillus sp.*
- *Citrobacter sp.*
- *Enterobacter sp.*
- *Microbacterium paraoxydans* (Fig. 2)
- *Ochrobactrum anthropic*
- *Pseudomonas chlororaphis* (Fig. 3)
- *Pseudomonas fluorescens* (Fig. 4)
- *Pseudomonas putida* (Fig. 5)
- *Pseudomonas sp.*
- *Stenotrophomonas maltophilia* (Fig. 6)

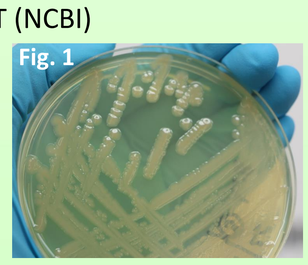
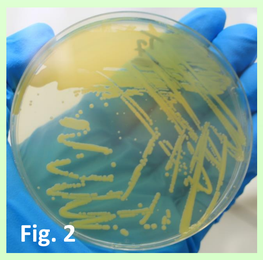


Fig. 1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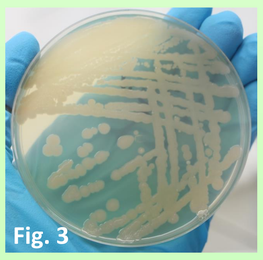
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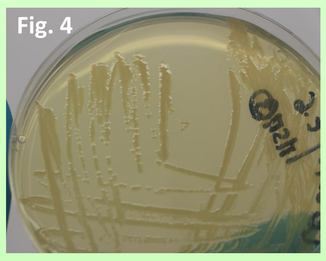
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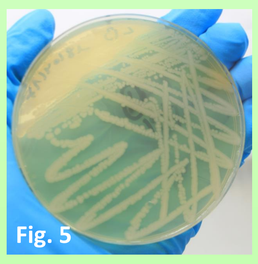
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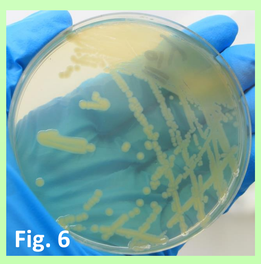


Fig. 6

Two fungal species still have to be identified

PAH DEGRADATION BY FUNGAL STRAINS

Studied fungal strains

- *Pleurotus ostreatus* MUCL 28518
- *Phanerochaete chrysosporium* MUCL 016186

Maximization of ligninolytic enzymes (LiP, MnP and LAC) production

Experimental design: culture medium, carbon source (*P. chrysosporium*: mannose, xylose, glucose ; *P. ostreatus*: mannitol, cellulose, glucose), carbon source concentration (1x, 2x)

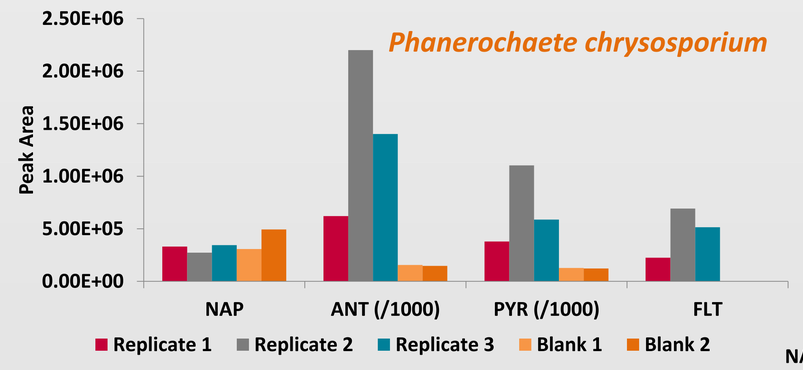
PAHs degradation experiments

- Best medium for enzyme production (*P. chrysosporium*: BSM medium, mannose 20 $g.L^{-1}$; *P. ostreatus*: BRM medium, mannitol 20 $g.L^{-1}$) + PAHs 0.1 $g.L^{-1}$ (NAP, ANT, PYR, FLT) + fungi
- Incubation 20 days, 28°C
- Blank: medium with poor enzyme production

Analytical method

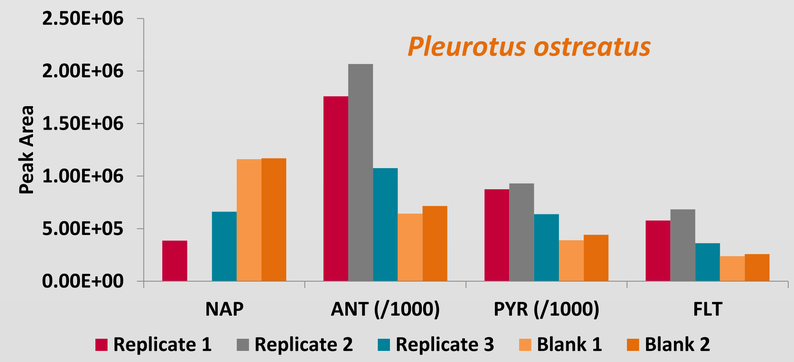
SPE purification (Supelco Envi-18 cartridges) before PAHs quantification by HPLC-PDA-FLD:

- Column: Agilent Zorbax Eclipse PAH (4.6 x 250 mm, 5 µm) with pre-column Agilent Zorbax Eclipse PAH (4.6 x 12.5 mm, 5 µm)
- Temperatures of column and samples: 25°C and 5°C respectively
- Elution: ACN/water mixture in gradient mode at a flow rate of 1.5 $mL.min^{-1}$



SPE procedure has to be improved: loss of NAP during the evaporation to dryness under N_2

NAP: naphtalene ANT: anthracene
PYR: pyrene FLT: fluoranthene



Preliminary results: no link between enzymes production and PAHs degradation

Future work:

- **Results verification: max. enzyme production/activity for degradation (≠ PAHs concentrations)**
- **Improvement of purification methods (SPE, LLE)**
- **Development of analytical methods for detection/quantification of PAHs/BTEX metabolites**
- **Influence of different parameters (temperature, surfactants, *etc.*) on fungal and bacterial degradation of PAHs/BTEX in liquid medium, slurry and soil column**

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