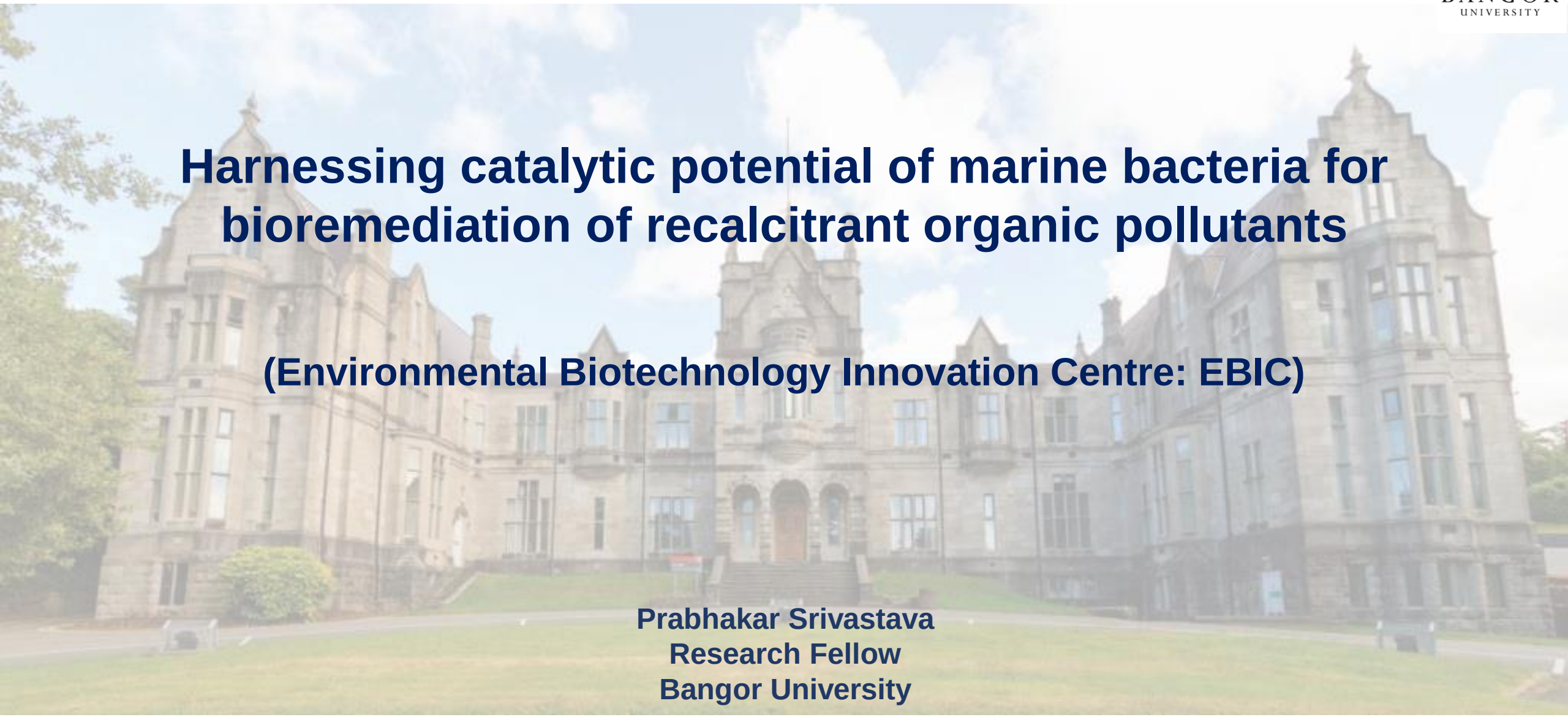


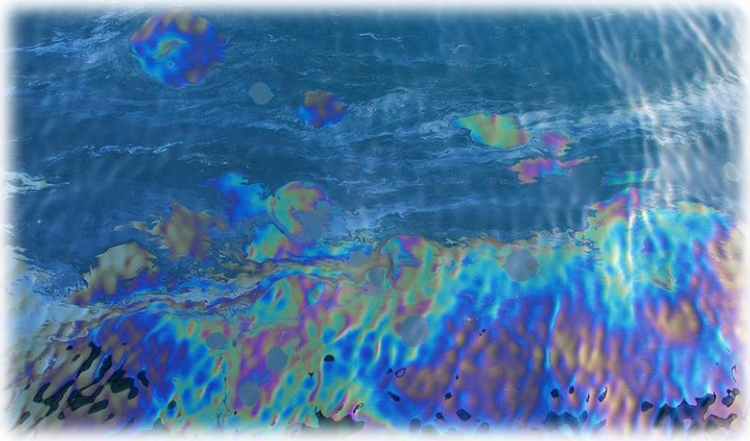
Harnessing catalytic potential of marine bacteria for bioremediation of recalcitrant organic pollutants

(Environmental Biotechnology Innovation Centre: EBIC)

Prabhakar Srivastava
Research Fellow
Bangor University

Bioremediation of targeted environmental pollutants

- **700 - 800 million tons of hydrocarbons** - each year in oceans - combination of **natural seeps**, **accidental releases** from the petrochemical industry, and the **biological activity of cyanobacteria: extensive damage to the marine ecosystem**.



- Hydrocarbon-metabolising bacteria – metabolic capabilities – biotransform various organic compounds

- Biologically useful microorganisms – major impacts on ecosystem

- Apply Synthetic Biology to enhance water and waste management practices, promoting the principles of the circular economy. By integrating Synthetic Biology into existing systems, innovative solutions will be developed to improve resource recovery, reduce waste generation, and optimize water treatment processes, leading to more sustainable and efficient practices.

https://education.nationalgeographic.org/resource/marine-pollution/ ; https://www.whoi.edu/know-your-ocean/ocean-topics/how-the-ocean-works/seafloor-below/natural-oil-seeps/

Tools and Objectives

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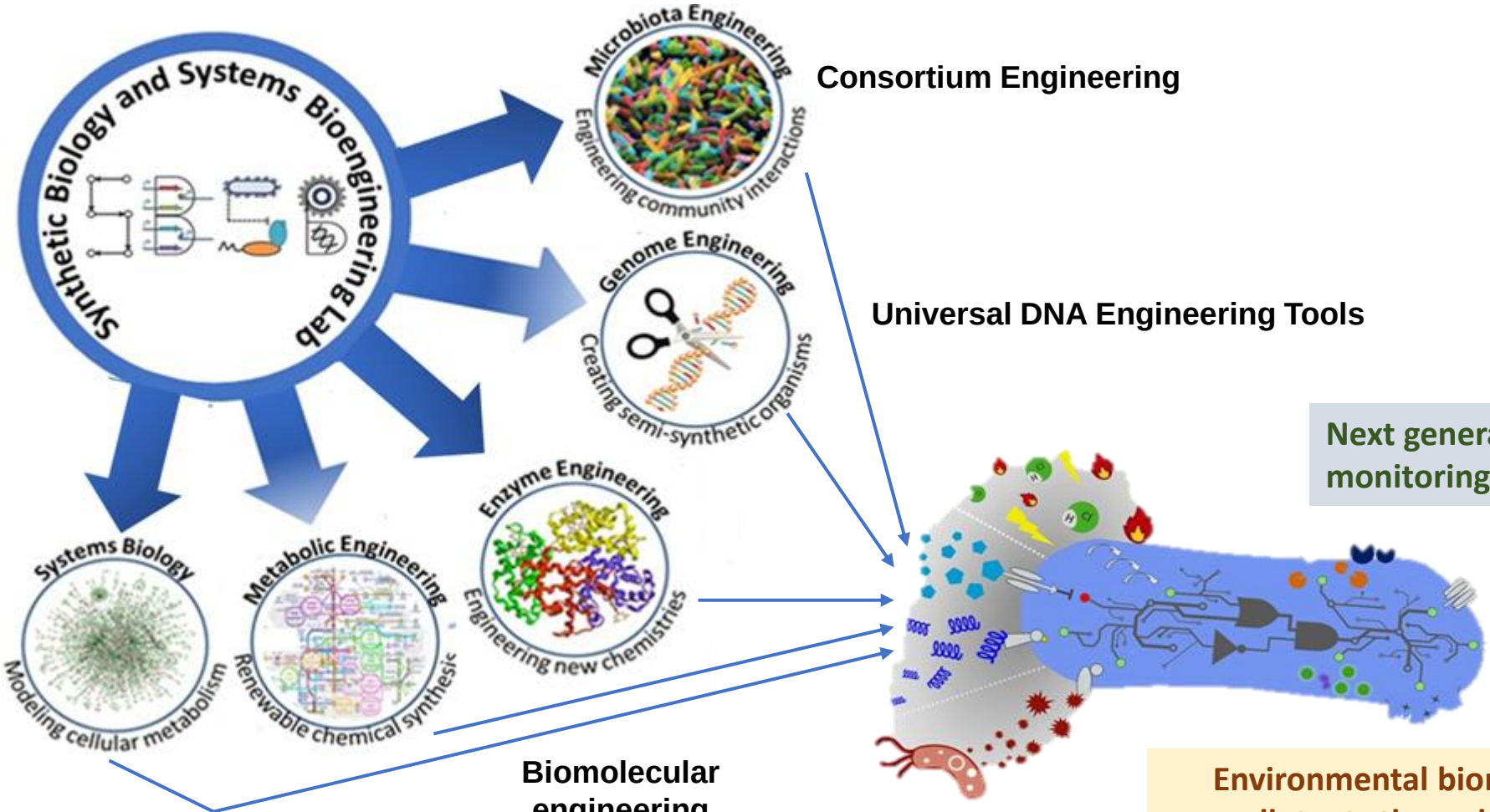

Consortium Engineering

Universal DNA Engineering Tools

Biomolecular engineering

Data Science/mind map systems biology

Next generation biosensors for environmental monitoring and surveillance

Environmental bioremediation of targeted environmental pollutants through biosequestration and biodegradation

Enhanced biofilm performance for bioengineered processes

Bioremediation of targeted environmental pollutants

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Engineering microorganisms to degrade recalcitrant organic pollutants and enhance natural remediation processes

Challenges:

- **Substrate complexity**
- **System robustness**
- **Genetic manipulation**
- **Consortia design**
- **Scale up**

Part I: Identification, isolation and optimisation of microorganisms, their enzymes and products (biosurfactants) to target specific pollutants (e.g. hydrocarbons, plastics and PFAS) degradations.

Part II: Genetically modifying and enhancing the biodegradation capabilities of strains known for hydrocarbon degradation including:

- ***Alcanivorax***
- ***Thalassolituus***
- ***Oleispira***
- ***Oleiphilus***
- ***Marinobactor***
- ***Cycloclasticus***

Enzymes for degrading recalcitrant organic pollutants

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- Hydrocarbons (alkanes) and fluorochemicals
- Carbon oxygenation and defluorination (C-H and C-F bonds)
- Phenols, industrial dyes

Metal-containing peroxidases, peroxygenases, monooxygenases, laccases and dehalogenases:

1. **Benchmark enzymes:** (P450 BM3, wt and 9-10A, HRP, **AbAlkB**).

2. **Peroxidases**: **23** proteins identified, cloned and purified.

3. **Unspecific peroxygenases:** (**16** bacterial peroxygenases cloned and purified, **3** fungal UPOs cloned).

4. **LPMO: 13** proteins cloned.

5. **Laccases: 3Cu^{2+} cofactor: 11** proteins cloned and purified.
   2Cu^{2+} cofactor thermostable: 26 enzymes cloned.

6. **Hydrolytic defluorinases and dehalogenases: 21** proteins cloned.
   2 proteins with split GFP+ leader peptide cloned for mutant-libraries generation.

Enzymes for degrading recalcitrant organic pollutants

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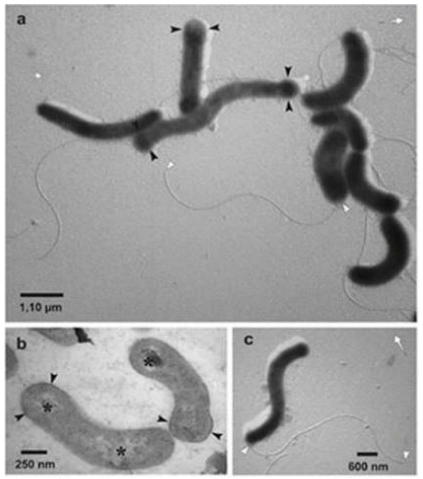
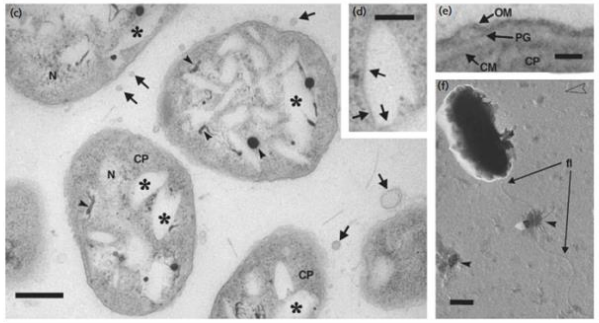
‘Obligate’ hydrocarbonoclastic bacteria: OHCB

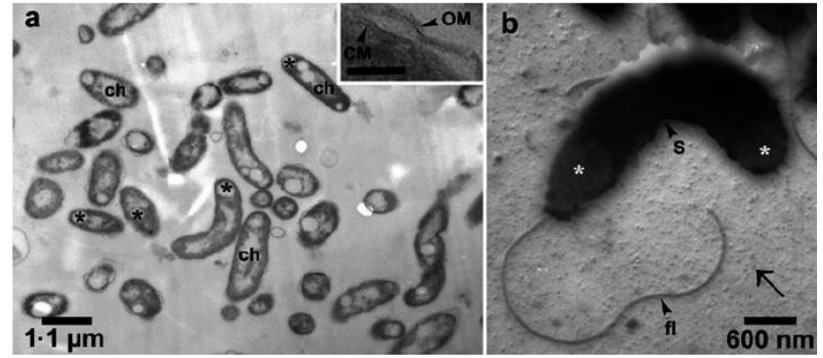


Oleispira antactica RB-8^T 4.2 Mb

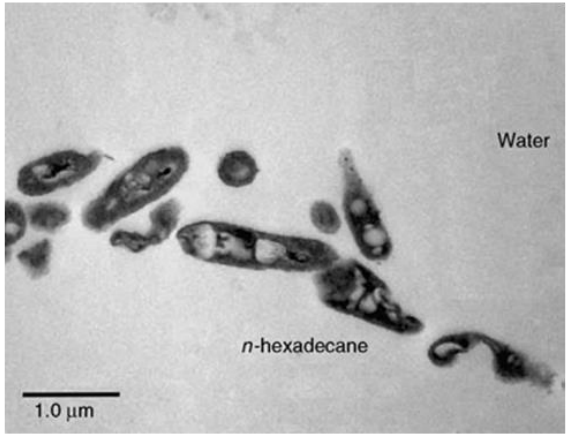


Oleiphilus messinensis ME-102^T *c*a. 6 Mbp

- Hydrocarbon degradation specialists.
- Unable to utilise sugars, amino acids etc.
- Degradation of linear & branched **C6-C24 alkanes**.
- Mechanism for hydrocarbon utilization is not fully understood.



Thalassolituus oleivorans Mil-1^T 3.8 Mbp



Alcanivorax borkumensis SK2^T 3.1 Mbp

Enzymes for degrading recalcitrant organic pollutants

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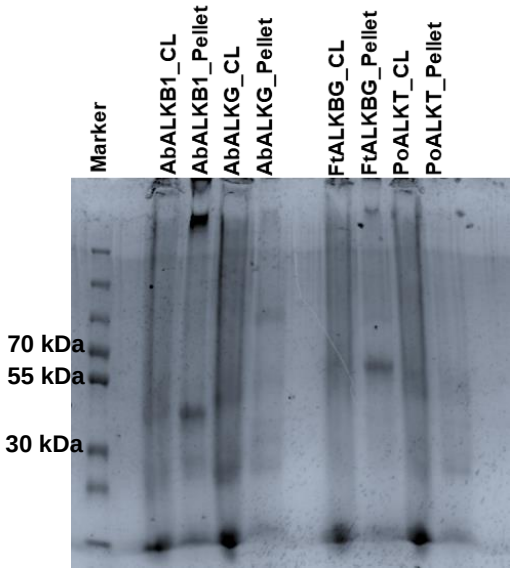
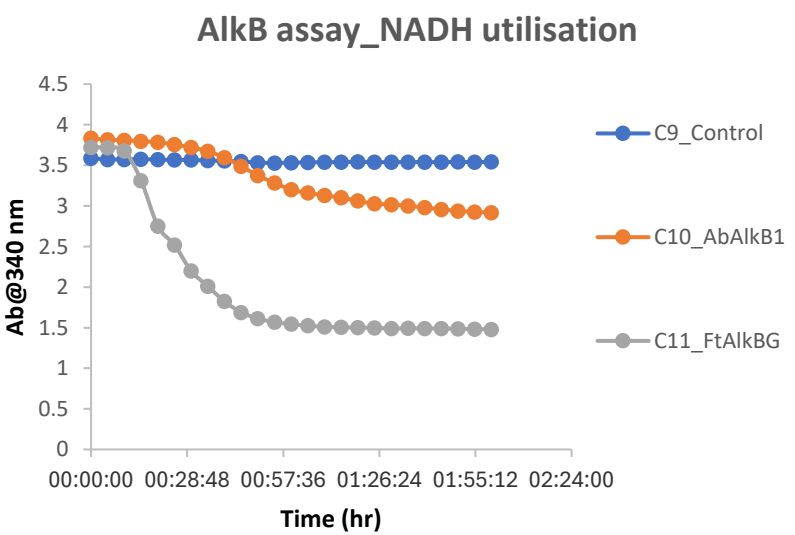
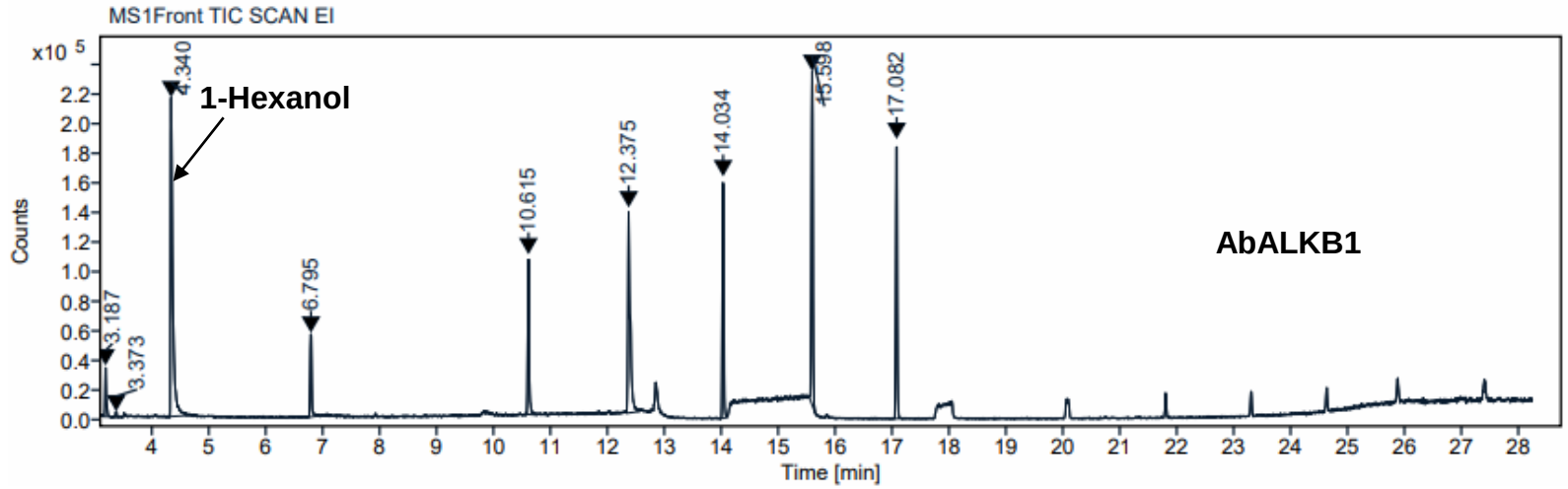
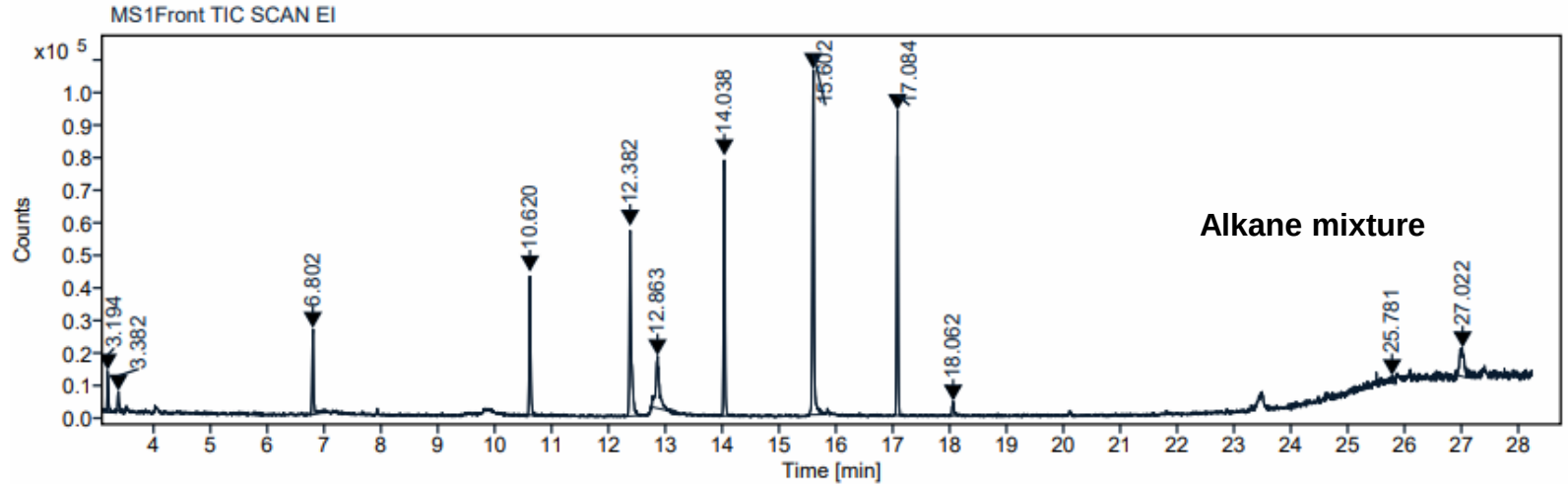
1. *Alcanivorax borkumensis* Alkane monooxygenase (AbALKB1)
2. *Alcanivorax borkumensis* Rubredoxin (AbALKG)
3. *Pseudomonas oleovorans* Rubredoxin reductase (PoALKT)









Enzymes for degrading recalcitrant organic pollutants

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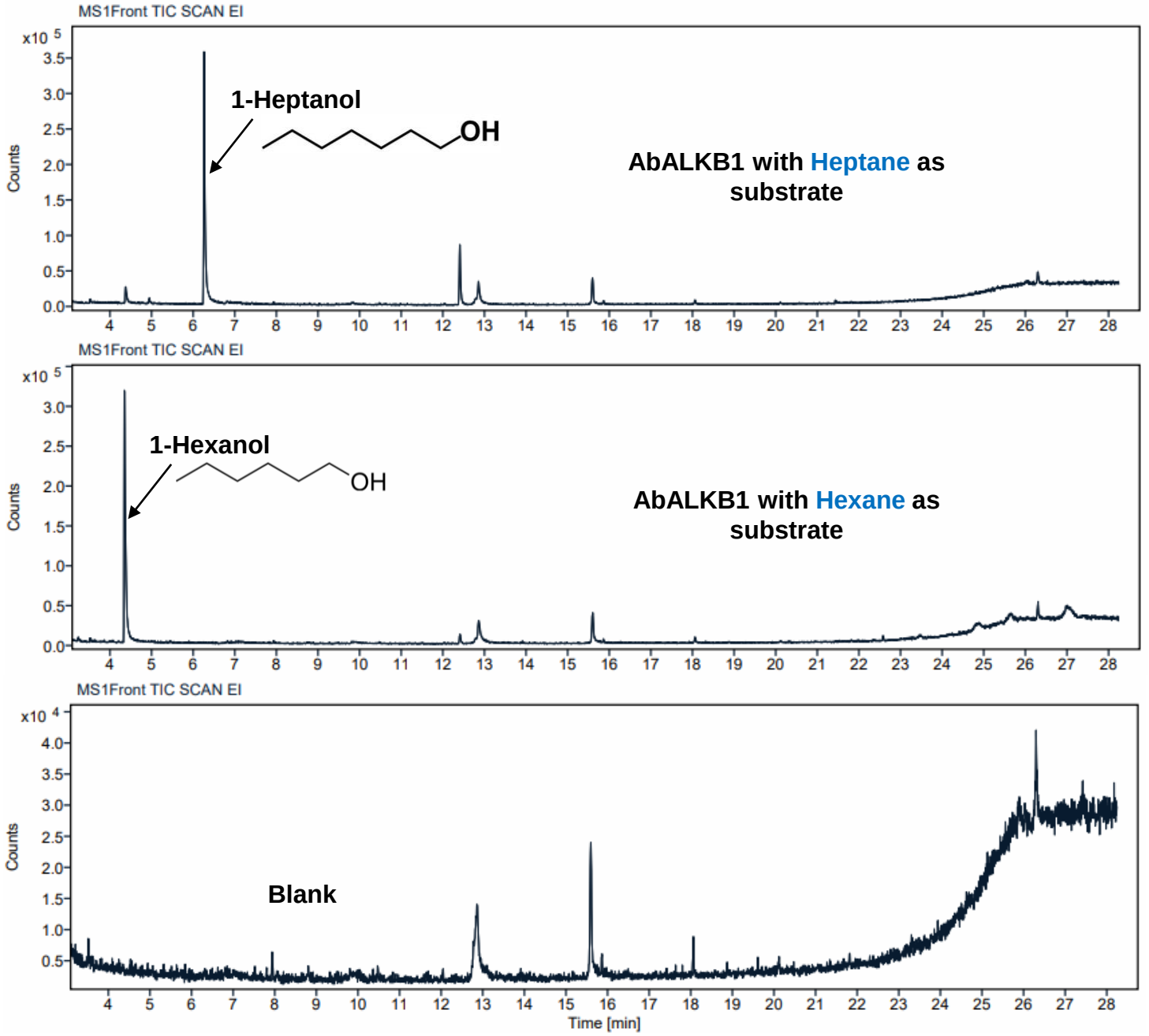
1. ***Alcanivorax borkumensis*** **Alkane monooxygenase (AbALKB1)**
2. ***Alcanivorax borkumensis*** **Rubredoxin (AbALKG)**
3. ***Pseudomonas oleovorans*** **Rubredoxin-reductase (PoALKT)**

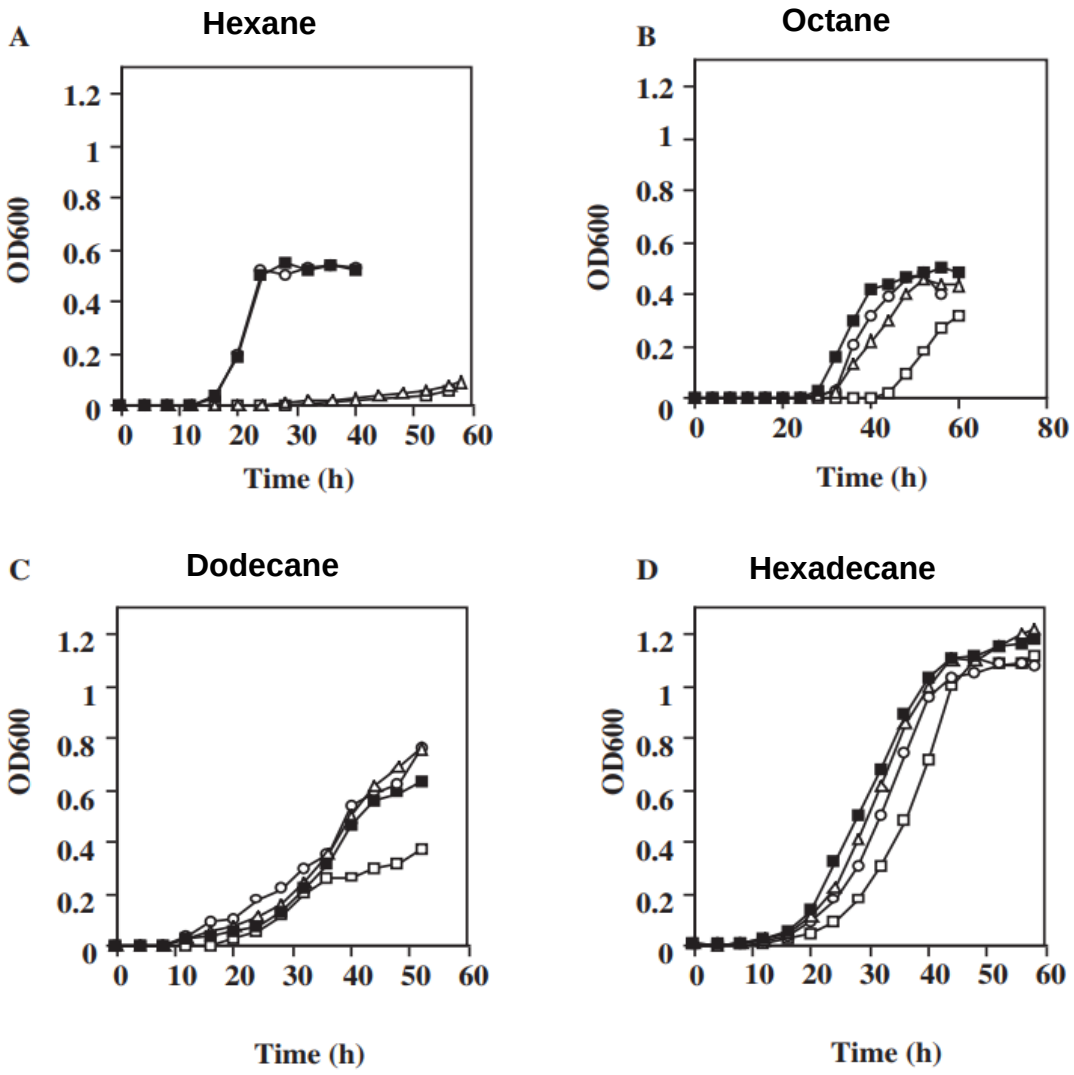
Hydrocarbons screened:

- Hexane
- Heptane
- Octane
- Decane
- Dodecane
- Tridecane
- Tetradecane
- Hexadecane



- ❑ Very little activity observed with **Octane**.
- ❑ No activity observed with **decane** and or larger chain length.

Enzymes for degrading recalcitrant organic pollutants



■ *A. Borkumensis* SK2 wild type

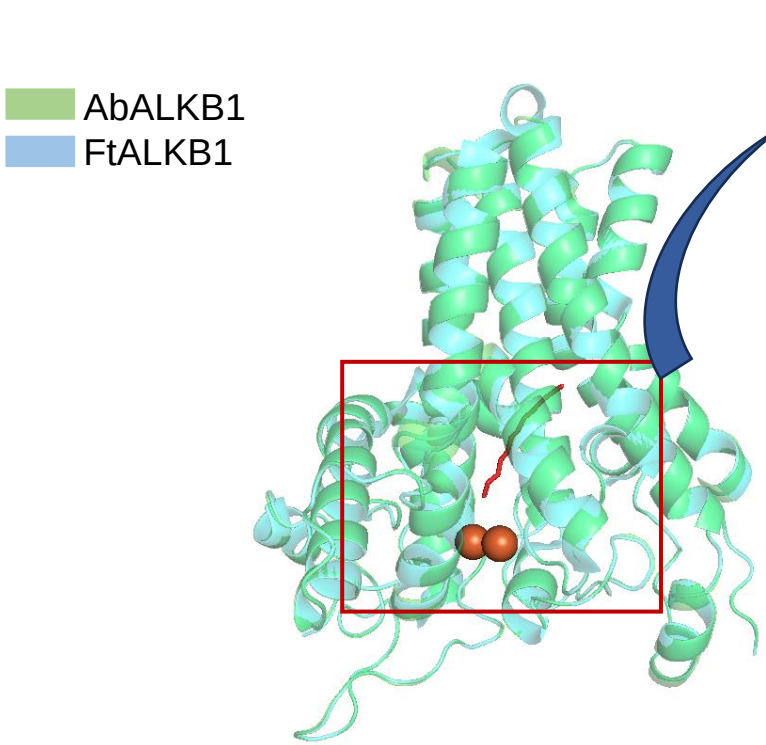
△ *A. Borkumensis* SK2 **ALKB1** deleted

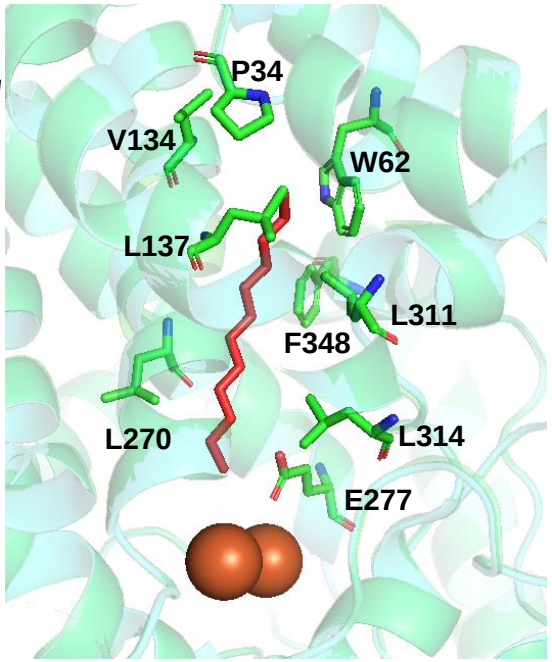
○ *A. Borkumensis* SK2 ALKB2 deleted

□ *A. Borkumensis* SK2 ALKB1 and ALKB2 deleted

Hara et al., Environmental Microbiology (2004) 6(3), 191–197

Enzymes for degrading recalcitrant organic pollutants





Active site pocket

Residues selected for mutagenesis

1. **W60V/A**
2. **L270A**
3. **E277L/A/Q**
4. **L311A**
5. **L314A**
6. **F348V/A**
7. **W60V/L270A/L311A/L311A**

- Engineering of active site pocket of AbALKB1 to broaden the substrate scope.
- Engineered variant will be incorporated into *Alcanivorax borkumensis* for better performance for bioremediation.

Enzymes for degrading recalcitrant organic pollutants

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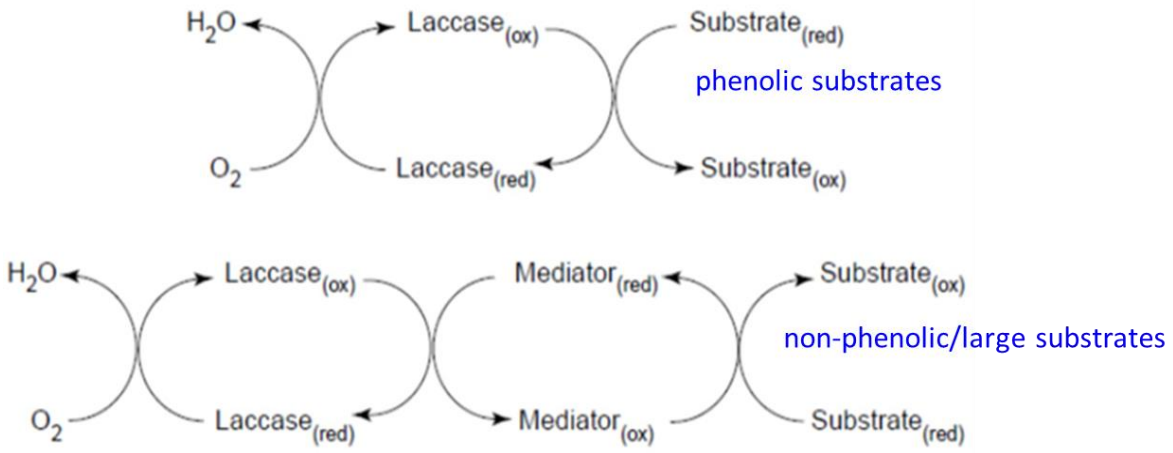
Bacterial Laccases
(24 soluble proteins in Bangor's collection)

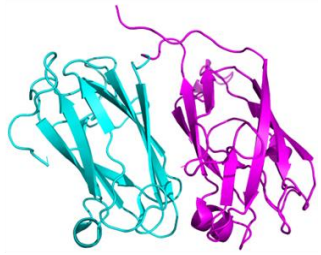
Broad substrate range: phenols, aromatic and aliphatic amines, petroleum hydrocarbons.



IPR001117

Laccase CotA from *B. subtilis*

IPR045704



Small laccase SC6420 from *S. coelicolor*

Laccase TSTM2506 from *Salmonella*

Enzymes for degrading recalcitrant organic pollutants

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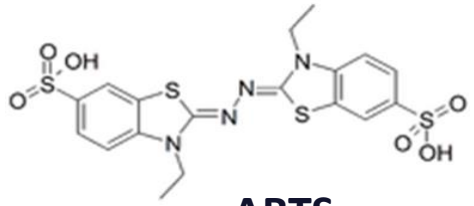
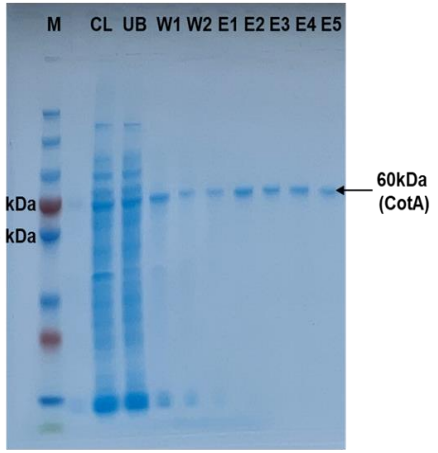
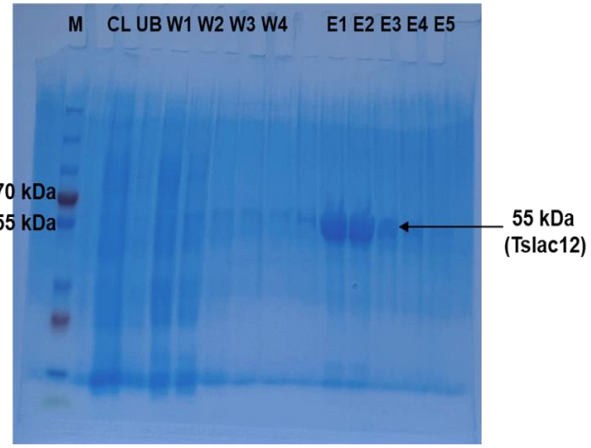
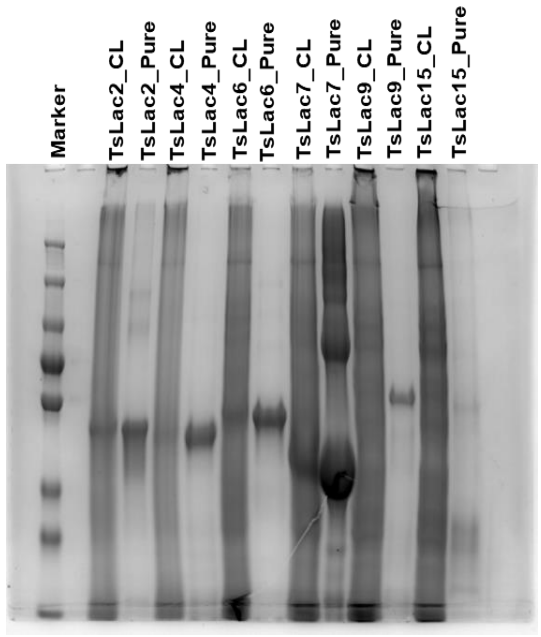
Bacterial Laccases:

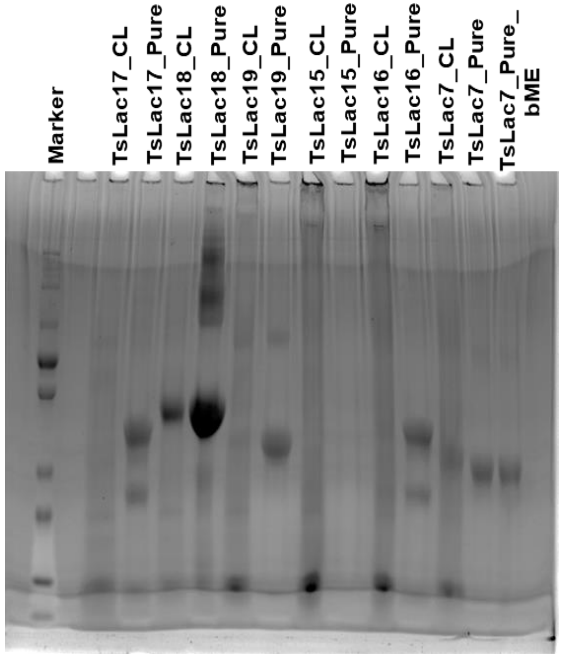


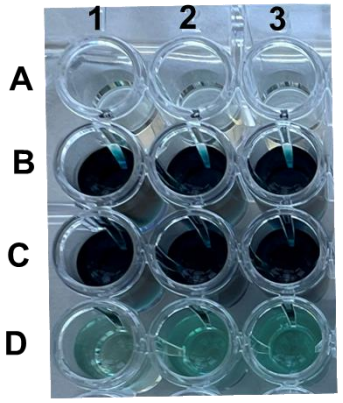
ABTS





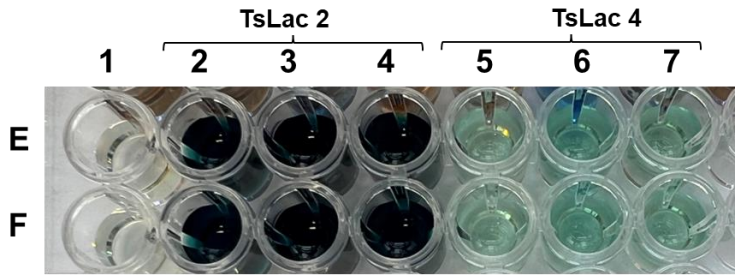




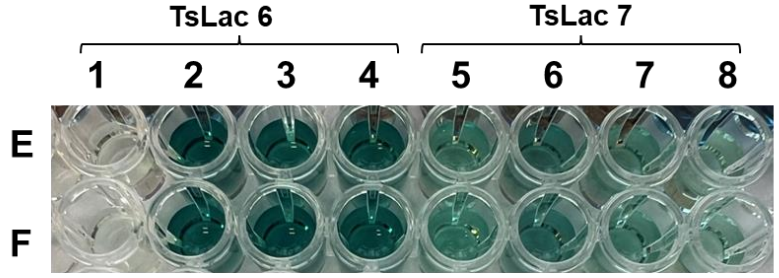


Legends:
A1, 2 and 3: Substrate (ABTS) blank, without enzyme
B1, 2 and 3: CotA (Old purification) with ABTS
C1, 2 and 3: CotA (Fresh purification) with ABTS
D1, 2 and 3: TsLac12 with ABTS

B1, C1 and D1: 3µg purified protein
B2, C2 and D2: 6µg purified protein
B3, C3 and D3: 12µg purified protein

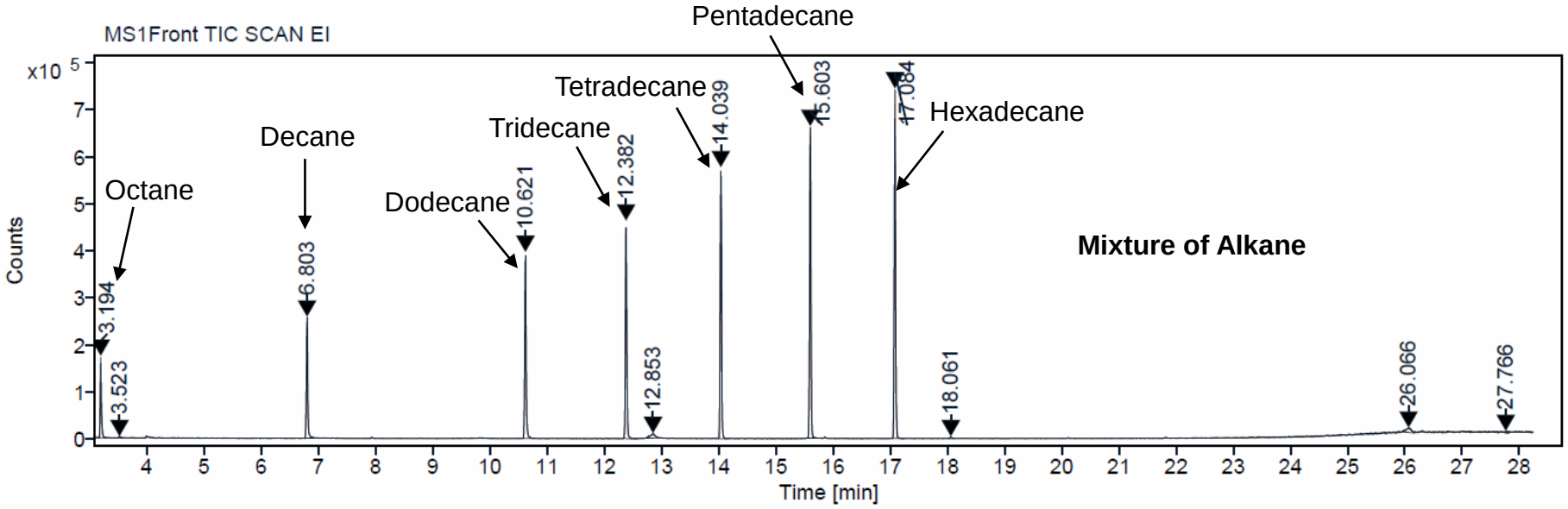


Legends:
E1, F1: Substrate (ABTS) blank, without enzyme
E and F 2, 3 and 4: TsLac2: Elution fractions with ABTS
E and F 5, 6 and 7: TsLac4: Elution fractions with ABTS



Legends:
E1, F1: Substrate (ABTS) blank, without enzyme
E and F2, 3 and 4: TsLac6: Elution 1, 2 and 3 with ABTS
E and F 5, 6, 7 and 8: TsLac7: Elution 1, 2 and 3 with ABTS

Enzymes for degrading recalcitrant organic pollutants



Screening of laccases against **aliphatic** and **aromatic hydrocarbons** and **Per- and polyfluoroalkyl substance** (PFAS).

Acknowledgements

Prof. Peter N. Golyshin
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THANK YOU