DRIP - Diatom-based Remediation via Immobilized Proteins

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Abstract

Water quality is threatened by micropollutants and microplastics. Since conventional wastewater treatment plants are not able to effectively remove these micropollutants, they end up in nature where they can exert negative effects on various organisms. Our project aims to tackle the challenge of micropollutants by developing a **diatom-based remediation platform**. In using a method called Live-Diatom Silica Immobilization (**LiDSI**), we were able to engineer the diatom *Thalassiosira pseudonana* to express and *in vivo* immobilize the enzymes laccase and FAST-PETase into its silica cell wall for the very first time. These enzymes are able to **degrade major pollutants**: PET-microplastics and pharmaceuticals like diclofenac and sulfamethoxazole respectively. To ensure biological safety in a future application, cells are lysed and the biocatalytically active biosilica isolated to be employed as a **filter material**. Being based on a unicellular, phototrophic organism, our remediation platform may present a renewable, cost-efficient and scalable solution to micropollution.

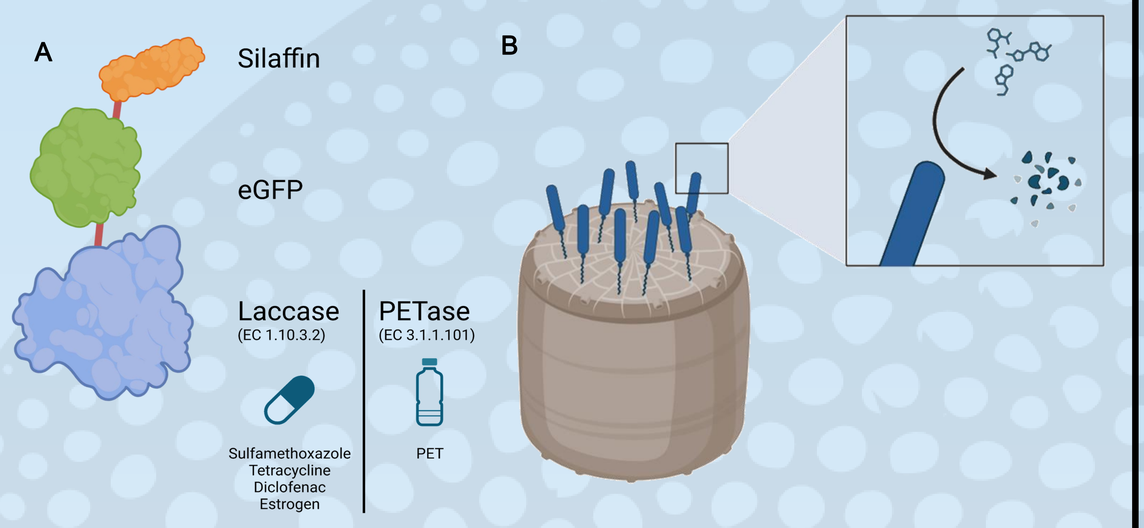

Figure 1 Illustration of fusion protein and immobilized enzymes on *Thalassiosira pseudonana*: Fusion protein consisting of anchor protein (silaffin), eGFP linker and enzyme (Laccase, FAST-PETase) (A). Schematic illustration of on *Thalassiosira pseudonana* immobilized enzyme, degrading a pollutant (B).

Methods

Transformation of *Escherichia coli* BL21 DE3 as
- **Negative control**
- **Heterologous production host of fusion protein**

Transformation of *Thalassiosira pseudonana*
- **Expression and immobilisation of fusion protein on algae surface**

Cell lysis with ultra sonication
- **Secure lysation of genetically modified organism**
- **Harvesting of biological active cell wall**

HPLC & photometric assays – pNPA, ABTS assay
- **Activity screening for immobilized enzyme**

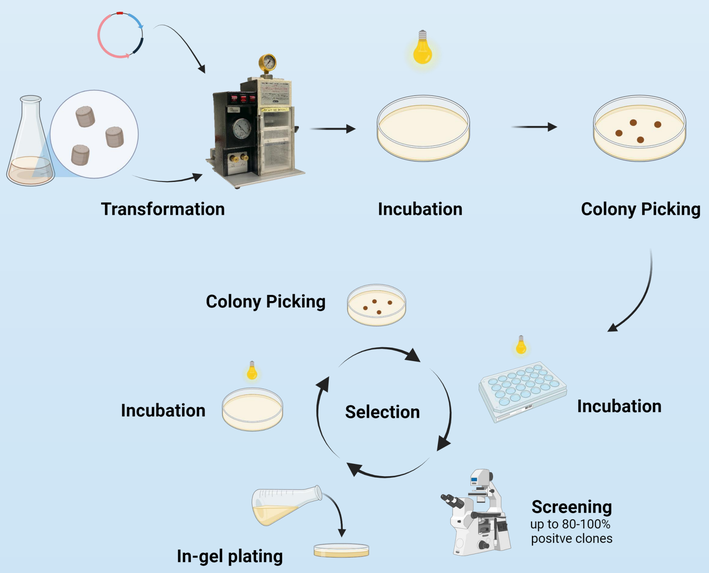

Figure 2 Transformation and selection process of *Thalassiosira pseudonana*: Co-transformation of plasmid pTpNR_T8, containing the sequence of interest, and pTpfc/nat, followed by light incubation (6-8 days). Subsequent steps include colony picking, culturing, and further screening by light microscopy for eGFP signal detection. The best candidates are selected for the next screening cycle.

Results

Successful transformation of *Thalassiosira pseudonana* with
- Bacterial laccase from *E. coli* (EcoL) and *Bacillus pumilus* (BpuL)
- FAST-PETase

Expression validated by observation of eGFP under the microscope

Purified, genetically modified cell walls show **activity for laccase EcoL**

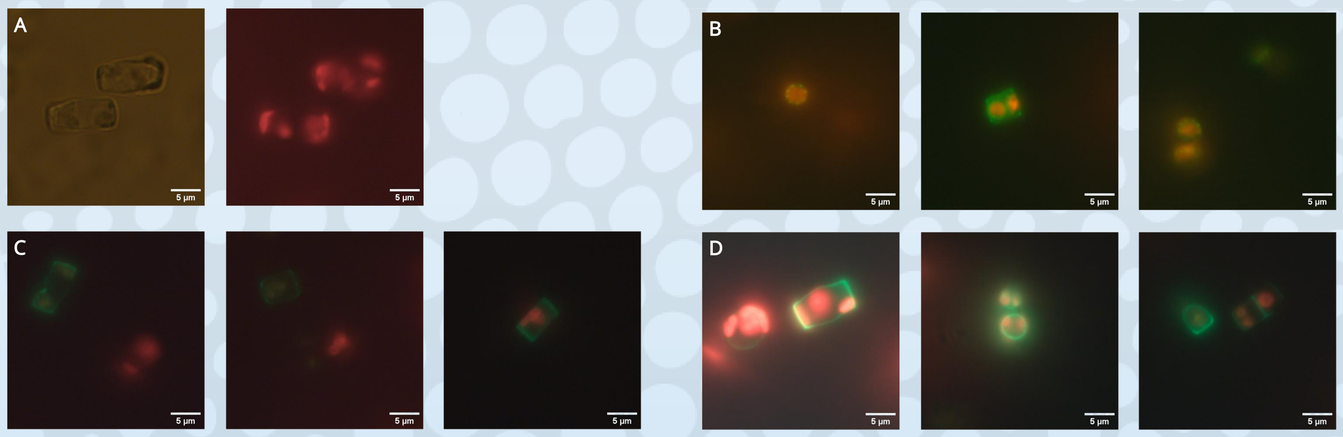

Figure 3 Microscope image analysis of *Thalassiosira pseudonana* transformants: Wildtype under bright field with chloroplast autofluorescence (A), expressing tpSil3_eGFP_EcoL (B), tpSil3_eGFP_BpuL (C) and tpSil3_eGFP_Fast-PETase (D). eGFP signal in green and chloroplast autofluorescence in red.

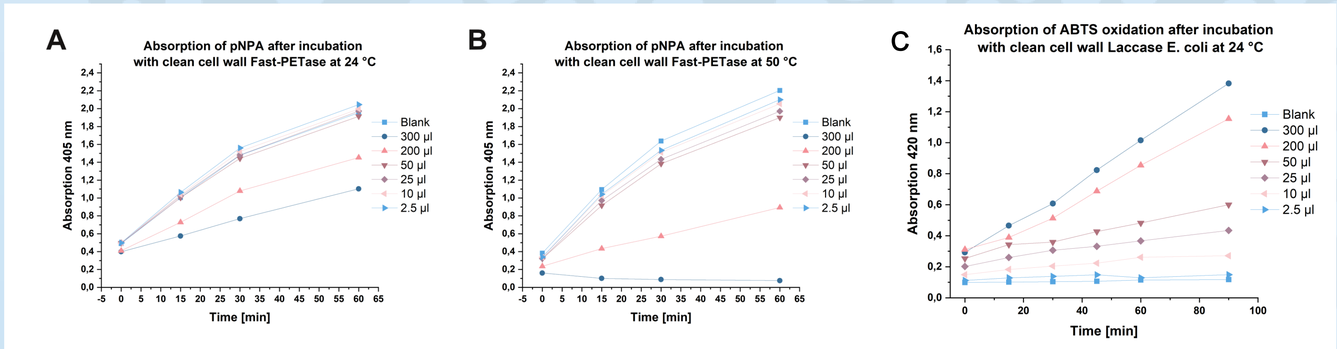

Figure 4 Photometric pNPA and ABTS assay for immobilized enzymes: Absorption of pNPA after incubation with cell wall immobilized Fast-PETase at 24 °C (A) and 50 °C (B). The absorption change was measured at 405 nm over 60 minutes. Absorption of ABTS oxidation after incubation with cell wall immobilized laccase EcoL at 24 °C (C). The absorption change was measured at 420 nm over 90 minutes. These experiments were conducted individually.

Outlook

Further characterization of immobilized laccases and FAST-PETase
- HPLC assays with diclofenac, sulfomethoxazole and PET-microplastics
- Establishing measurement assays for FAST-PETase characterization

Enhancing protein expression in *Thalassiosira pseudonana*
- Testing of different silaffin anchor proteins and promotors

Implementation of different selection marker
- Replacement of antibiotics with auxotrophic marker

Website

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