A microbial electrochemical technology to detect and degrade organophosphate pesticides

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Supplemental Information

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1. Strain Engineering

\textit{p-NP-responsive strains}: The DmpR gene sequence and the \textit{PDmpR} sequence were cloned into the same vector. DmpR was placed under the control of the LacI promoter to ensure constitutive expression, while the \textit{PDmpR} sequence was placed in front of the gene of interest (either sfGFP or CymA). The cloning was performed using a four-part Gibson assembly strategy.\textsuperscript{1} The vector pieces were amplified by PCR from plasmids pCD7sfGFP and pCD26r4 (these plasmids were gifts from the Keitz Lab). The pCD plasmids have a ColE1 origin of replication, kanamycin resistance, a lacI repressor constitutively expressed, and either sfGFP or CymA under the control of an IPTG-inducible promoter. We designed primers such that DmpR would replace the lacI repressor and \textit{PDmpR} would replace the IPTG-inducible promoter. Thus, each Gibson Assembly reaction had four fragments: 1) the vector containing the gene of interest, origin of replication, antibiotic resistance, etc., 2) \textit{PDmpR}, 3) \textit{PlacI}, and 4) DmpR. PCR with Phusion High-Fidelity DNA Polymerase (NEB: M0530) in HF buffer was used to generate DNA fragments. The PCR parameters were: initial denaturation at 98 °C for 30 s followed by thirty-five cycles of 1) 98 °C for 10 s, 2) 61 °C for 30 s, and 3) 72 °C for 2 mins 30 s, followed by a final extension at 72 °C for 10 minutes. PCR products were run on a 1.6% agarose gel at 100 V for 30 minutes. Desired fragments were gel extracted using the Zymoclean Gel DNA Recovery Kit (Zymo Research). Gibson Assembly reactions were performed in a 20 µL reaction volume using a 1:3 molar ratio of vector to insert. Gibson Assembly master mix was prepared in house and contained ISO buffer, T5 exonuclease, Phusion polymerase, and Taq DNA ligase. Reactions were performed at 50 °C for 1 hour. After incubation, 10 µL of Gibson assembly reaction was transformed into chemically competent DH5α \textit{E. coli} and plated on LB-agar kanamycin plates to select for positive transformants. Sequence verification of correctly cloned plasmids was performed as described above. After sequence verification, plasmids were transformed by electroporation into the appropriate strains of \textit{S. oneidensis}. Electroporation protocol was adapted from Dundas \textit{et al.}\textsuperscript{2} In short, \textit{S. oneidensis} cells were made competent by washing 3x and resuspending in 10% glycerol solution. After addition of ~100 ng DNA, electroporation was performed in a BTX Harvard Apparatus ECM 399 Electroporation System using 1 mm electroporation cuvettes at 1250 V. Cells are recovered for 2 hours by shaking at 30 °C at 200 rpm and plated on LB-kanamycin (25 µg/mL) plates for selection. The plasmid containing \textit{PDmpR>sfGFP} was transformed into wildtype \textit{S. oneidensis} MR-1 (gift from the Keitz Lab), and the plasmid containing \textit{PDmpR>cymA} was transformed into the genetic knockout strain \textit{S. oneidensis} MR-1 Δ\textit{cymA} (gift from the Keitz Lab).

2. Colorimetric Assay for Organophosphate degradation using lyophilized OPH-\textit{E. coli}

The colorimetric assay was performed in a 96-well plate using a multi-mode microplate reader (BioTek, Winooski, VT). Lyophilized OPH-\textit{E. coli} cells in phosphate citrate (PC, 50 mM Na\textsubscript{2}HPO\textsubscript{4}, 9.5 µM citric acid monohydrate, pH = 8.0) buffer were reconstituted in sterile water and diluted to a final \textit{OD}	extsubscript{600} of 0.02 in 200 µL PC buffer. Just before the measurement, organophosphates (OP), including paraoxon and parathion, were added from 100% MeOH stock solutions to the experimental wells at different concentrations keeping the final MeOH
concentration at 0.1% (v/v). For paraoxon-methyl, the stock solutions were made of 10% MeOH in water, making the final MeOH concentration in the experimental wells 0.01% (v/v). Controls with only OPH-E. coli cells and only the corresponding OP were also included. P-NP production was monitored by measuring absorbance at 400 nm over 2 hours at 27 °C. Standard curves generated from known concentrations of p-NP were used to convert the absorbance values to p-NP concentrations. To determine the effect of enzyme concentration on OP degradation, absorbance at 400 nm was measured at a constant paraoxon concentration (25 µM) and varying cell concentrations starting from final OD600 0.04 and serially diluting six times by half. Parameters of enzyme kinetics were determined by fitting the data to the Michaelis-Menten equation using OriginLab data analysis software (Northampton, MA).

3. GFP Fluorescence Assay for Monoculture and Co-culture Experiments

Engineered S. oneidensis strain was grown overnight for 18-20 hours in 50 mL LB supplemented with 25 µg/mL kanamycin at 30 °C and 200 rpm from 25% frozen glycerol stocks (stored at -80 °C). The pre-culture was diluted in 30 mL Terrific Broth (TB) supplemented with potassium phosphate buffer (17 mM KH2PO4, 72 mM K2HPO4), and 25 µg/mL kanamycin to 0.1 OD600 and incubated at 30°C at 200 rpm until the OD600 reached 0.8.

To determine the effect of p-NP on bacteria, the culture was split into 10 tubes and induced with increasing concentrations of p-NP ranging from 0 to 200 µM. The incubation was continued at 30 °C and 200 rpm for 24 hours. Cells were pelleted from 100 µL of the cultures by centrifugation at 14,265 × g for 2 min. The supernatant was discarded, and the cells were washed twice by centrifugation and resuspension with PBS (pH 7.4). The cells were then pipetted in a 96-well plate at 1/10th dilution in PBS and the fluorescence was measured in a microplate reader (BioTek, Winooski, VT) at excitation λ 485 nm and emission λ 510 nm.

For co-culture measurements, lyophilized OPH-E. coli cells in PBS were reconstituted in sterile water and inoculated to OD600 0.02 with engineered S. oneidensis culture in TB at OD600 0.8. OPs at different final concentrations were added to the co-cultures and incubated at 30°C, 200 rpm for 48 hours. Periodically, 100 µL of cell aliquots were withdrawn from the cultures; cells were pelleted and washed twice by centrifugation and resuspension in PBS at 14,265 × g for 2 minutes, and the fluorescence was measured as described above. In both monoculture and co-culture assays, the fluorescence was normalized with OD600.

4. Bioelectrochemical Measurements

S. oneidensis (engineered or wild-type) was grown overnight for 18-20 hours in 50 ml LB supplemented with 25 mg/mL kanamycin at 30 °C and 200 rpm from 25% frozen glycerol stocks (stored at -80 °C). The cells were pelleted by centrifugation at 11,940 × g for 5 min. The supernatant was discarded, and the cells were washed twice by centrifugation and resuspension in M1 minimal buffer adapted from previous reports containing 50 mM sodium salt of PIPES buffer, 28 mM ammonium chloride, 1.34 mM potassium chloride, and 4.35 mM sodium phosphate.
monobasic. After the final wash, the cells were resuspended in 1 mL M1 media and inoculated in bioreactors for electrochemical measurements.
5. Supplemental Figures

Figure S1: Production of $p$-NP over time by OPH-expressing *E. coli* at different cell densities represented as OD$_{600}$. Error bars represent SD for $n$=3 replicates.
Figure S2: Production of p-NP over time by OPH-expressing *E. coli* at different organophosphate concentrations for a) parathion and b) paraoxon-methyl. Effect of organophosphate concentration on the initial rate of p-NP production, fitted with Michaelis-Menten equation (red) for c) parathion and d) paraoxon-methyl. Error bars represent SD for *n=3* replicates.
**Figure S3:** GFP fluorescence normalized by OD$_{600}$ produced by p-NP responsive GFP-expressing *E. coli* strain in response to different concentrations of p-NP (blue) and paraoxon (light blue), and by wild-type *E. coli* cells with different p-NP concentrations (grey) after 24h induction. Error bars represent SD for *n=3* replicates.

**Figure S4:** GFP fluorescence normalized by OD$_{600}$ produced by engineered *S. oneidensis* in response to different concentrations of p-NP (red), dopamine (purple), hydroquinone (blue) and 4-methylcatechol (green) after 24h induction. Error bars represent SD for *n=3* replicates.
Figure S5: Raw data from representative chronoamperometry plots indicating the change in current density over time in bioreactors containing engineered *S. oneidensis* with 20 µM *p*-NP (dark blue), cells with 20 µM paraoxon (blue), cells without any inducer (light blue) and 20 µM *p*-NP without cells (grey).

Figure S6: Normalized GFP fluorescence produced over time by engineered cell mixtures (OPH-expressing *E. coli* with *p*-NP inducible *E. coli*) in the presence of different initial concentrations of paraoxon, ranging from 0 to 150 µM and co-culture with wild-type *E. coli* containing 50 µM paraoxon (grey curve). Error bars represent SD for *n=3* replicates.
Figure S7: Normalized GFP fluorescence produced over time by engineered co-cultures (OPH-expressing E. coli with p-NP inducible S. oneidensis) in the presence of different initial concentrations of OPs, ranging from 0 to 150 µM and co-culture with wild-type E. coli containing 50 µM organophosphate (grey) for a) paraoxon-methyl b) parathion c) malaoxon. Error bars represent SD for n=3 replicates.

Figure S8: Raw data from representative chronoamperometry plots indicating the change in current density over time in bioreactors containing engineered co-cultures with 20 µM paraoxon (dark blue curve), S. oneidensis with 20 µM paraoxon (blue curve), co-culture only (light blue curve) and 20 µM paraoxon without cells (grey curve).
**Figure S9**: Representative chronoamperometry plots indicating the change in current density over time in bioreactors containing engineered co-cultures with 20 µM paraoxon-methyl (red), 20 µM parathion (purple), 20 µM malaoxon (green).

**Figure S10**: a) Representative chronoamperometry plots indicating the change in current density over time in bioreactors containing engineered co-cultures with different concentrations of paraoxon, ranging from 0 to 20 µM. b) Effect of paraoxon concentration over the total charge accumulated by the co-culture in 24 h, fitted with a linear regression line from 5 – 20 µM (red).
References

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