

ELECTRONIC SUPPLEMENTARY INFORMATION

**Metagenomic discovery of a novel transaminase for valorization of  
monoaromatic compounds**

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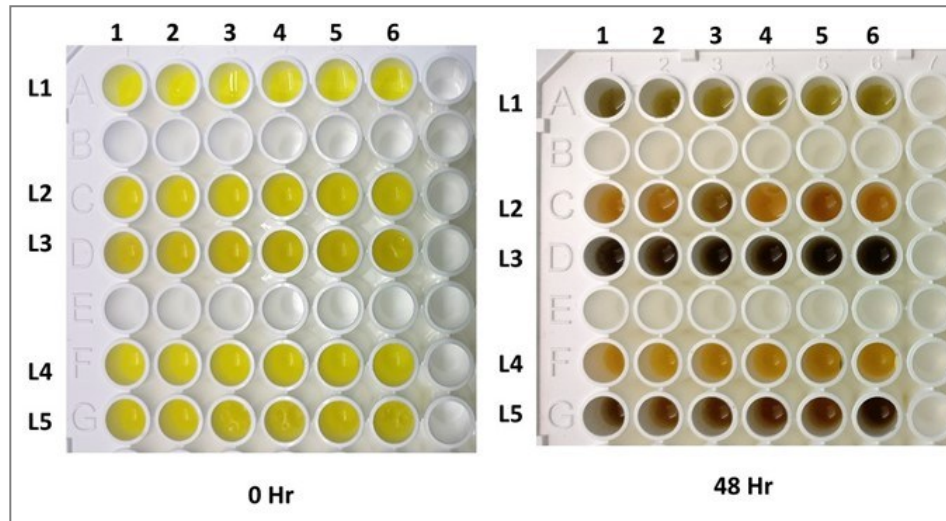
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**General information**

The solvents and chemicals are purchased from Sigma Aldrich, Alfa Aesar and SantaCruz biotech and were used without any further purification. HPLC analyses were performed on Perkin Elmer HPLC equipped with Flexar PDA plus multi wavelength detector and Chromera software. The mobile phase consistS of 10mM of ammonium acetate and acetonitrile ran in gradient mode using Water atlantis C18 column (5 $\mu$ , 4.6 mm x 250 mm). The gradient starts at 0 min with 60% of ammonium acetate and 40% of ACN, 10 min 100%ACN and to 60% of ammonium acetate and 40% of acetonitrile at 20 min. The standard Benzylamine,  $\alpha$ -methylbenzylamine, Benzaldehyde, and Acetophenone eluted at 3.6, 4.2, 7.7 and 8.1 min, respectively. GCMS analysis was performed by using Perkin Elmer Clarus 680 GC system equipped with Perkin Elmer Clarus SQ8T EI mass selective detector and Agilent HP5MS Capillary Column (Length 25m, Film thickness 0.20  $\mu$ m, Inner diameter 0.25 mm). Operating conditions involved TGC (injector), 250 °C; TMS (ion source), 200 °C; oven time program (T0 min), 50 °C; T2 min, 50 °C; T22 min, 300 °C (heating rate 15 °C min<sup>-1</sup>); and T25 min, 300 °C. The injector volume was 1  $\mu$ L.

## High-throughput screening

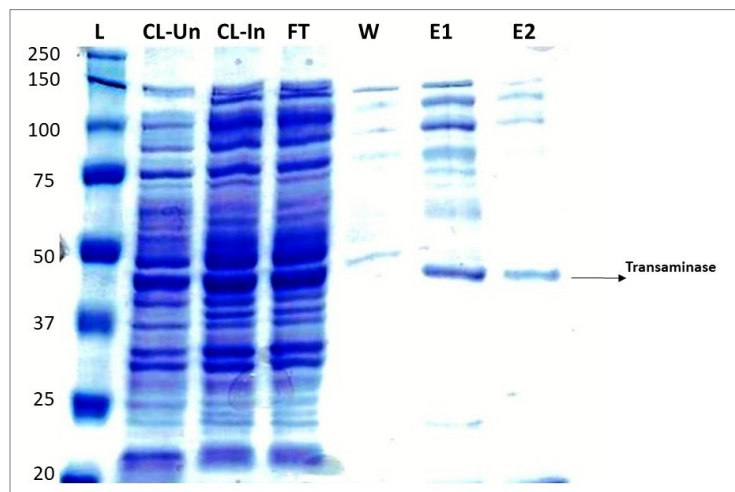
High throughput screening was performed on the fosmid library derived from soil samples, consisting of 3072 clones organized in 384 well microplates. Fosmid clones were replicated using a Qpix2 robotic colony picker (Genetix) in 384 well white microplates at an initial 55  $\mu\text{L}$  volume in LB media supplemented with chloramphenicol (12.5  $\mu\text{g}/\text{mL}$ ) and L-arabinose (100 $\mu\text{g}/\text{mL}$ ). When considering the volumes to be dispensed, a total of 5 $\mu\text{L}$  volume was considered to have evaporated after 24 hours. Microplates were incubated at 37°C for 24 hours to provide initial growth to fosmid clones, and subsequently induced with reaction components benzaldehyde 5 mM, Ortho-xylylenediamine 25 mM, PLP 2 mM, supplemented with the same concentrations of chloramphenicol and L-arabinose. The microplates induced with substrates and cofactor were incubated at 30°C for an additional 48 hours. After 48 hours, the microplates were observed carefully to identify the wells with color change. Fosmid clones that showed significant colour change was identified and streaked on solid agar plate with chloramphenicol antibiotic selection marker. The single colony was picked from agar plate and fosmid DNA was extracted using Genejet Plasmid extraction kit. The fosmid clone was fully sequenced and annotated to identify the presence of TA expressing ORF.



**Figure S1:** Colorimetric assay in 96 well plate with identified TA from HT screen. L1: Control without amino acceptor 1, L2- pET15b-TA Whole cell (100  $\mu\text{l}$ ), L3- pET15b-TA crude cell lysate ( $\sim 0.5\text{mg} - 1\text{mg}/\text{ml}$ ), L4- S2A24 fosmid whole cell, L5- S2A24 crude cell lysate ( $\sim 0.5\text{mg} - 1\text{mg}/\text{ml}$ ) Reaction conditions: Benzaldehyde- 5mM, O-xylylenediamine – (Row 1-3) 5mM, (Row 4-6) 25mM, Pyridoxal phosphate: 2mM, Phosphate buffer- 100mM (pH 7.5), Temp- 30°C

## Cloning and expression

TA gene from identified fosmid clone was custom synthesized from Genewiz with *nde1* and *bamH1* cut sites and the operon was codon optimized for *E.coli*. The TA gene was PCR amplified from pUC57-Kan plasmid provided by genewiz with forward primer GTTACTTCCATATGACCCGGGACATTC and reverse primer GTTACTTCGGATCCTCATGTTGACTCCGCG. The PCR product was digested with *nde1* and *bamH1* and then ligated to *nde1* and *bamH1* digested pET15b to generate pET15b-TA construct. pET15b-TA was transformed into *E.coli*.BL21 (DE3) and transformed product was grown onto ampicillin (100µg/ml) agar plate. Single colony was picked from agar plate and incubated at 37 °C for overnight into 5mL LB media containing ampicillin (100µg/ml). The overnight culture was used to inoculate 10 mL LB media containing ampicillin (100µg/ml and incubated at 37 °C until the OD600 of 0.6 reached, at this point the culture was induced with 0.5mM IPTG and grown further at 25°C overnight. The cell were harvested by centrifugation and lysed with lysis buffer provided with Qiagen Ni-NTA spin column. The cell lysate was centrifuged and supernatant was collected and it was passed through Ni-NTA spin column. Fractions of flow through, wash, elution 1 and elution were collected and ran on SDS PAGE gel to confirm expression of TA. The gel picture is shown here in figure S2, which shows his-tag purified TA protein of approximate 41 KD size.



**Figure S2:** His-Tag purification of transaminase using Ni-NTA spin column. **L-** Ladder (MW, kD), **CL-Un:** Cell lysate of un-induced bacterial cell culture, **CL-In:** Cell lysate of induced bacterial cell culture, **FT:** Flow through, **W:** Wash, **E1:** Elution 1, **E2:** Elution 2

# Sequence alignment with different TAs

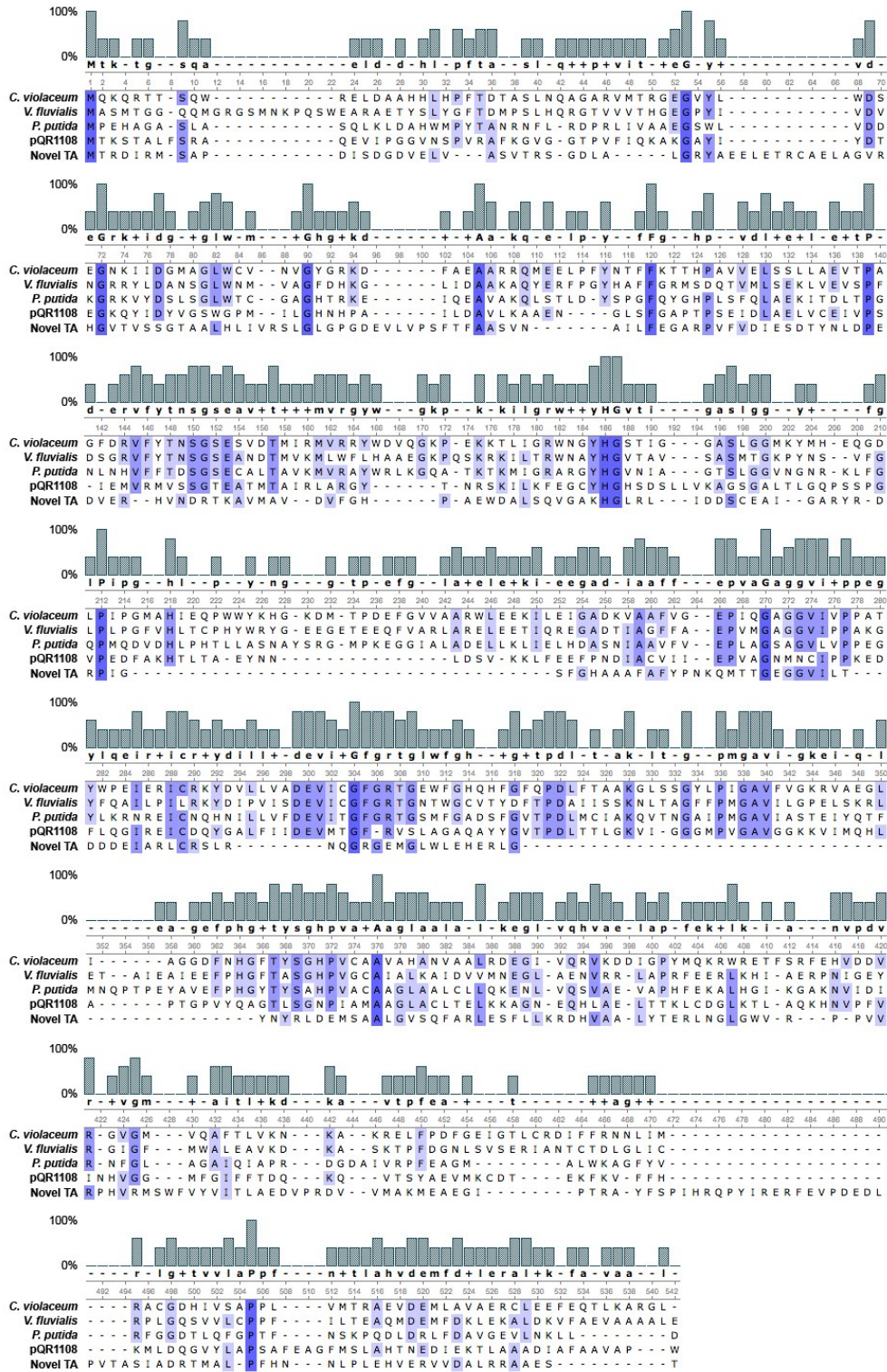


Figure S3. Multiple sequence alignment of pRT15-TA with different TA using CLUSTAL W multiple sequence alignment

## Biotransformation conditions

Ortho-xylylenediamine 25 mM, PLP 2 mM in potassium phosphate buffer (KPi 100mM, pH 7.5) and benzaldehyde (5mM) in DMSO and crude cell lysate (0.5-1 mg/ml) were added to 96 well white plate and final volume was make up with KPi (100mM, pH 7.5) to 200 $\mu$ l. The reaction was incubated at 30 °C for 48 hrs, at 200 rpm. The plate was sealed with sealing films to avoid evaporation of reaction mixture over period of reaction time. After 48 hrs the reaction was quenched with 500ul of methanol and the reaction mixture was centrifuged to remove precipitated protein and reaction component and the reaction mixture was analyzed using HPLC. In case of preparation of GC-MS analysis samples instead of adding methanol to quench reaction, after 48 hrs of reaction, reaction mixture was immediately extracted with ethyl acetate and was analyzed on GCMS.

## Calibration curves and HPLC curve for standards

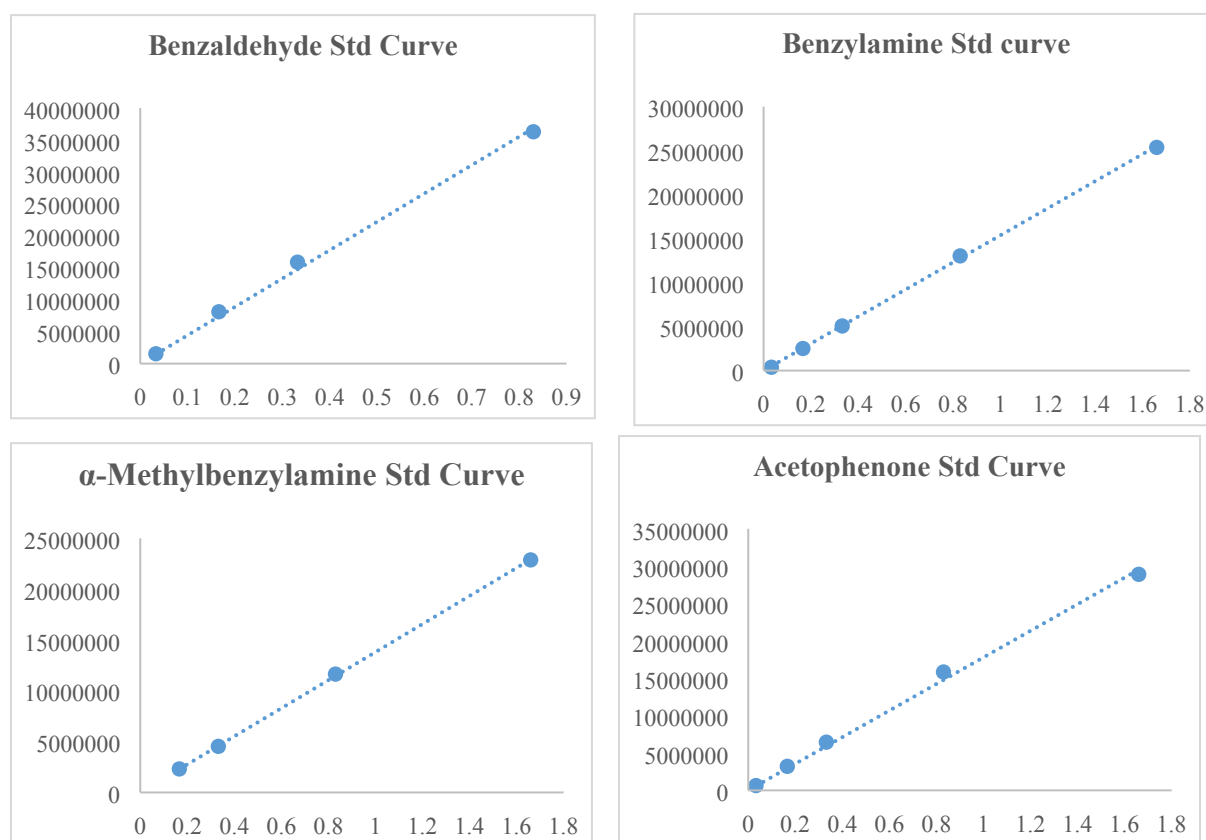


Figure S4. HPLC calibration curves