

## **Electronic Supplementary Material**

**Title: Characterization and protein engineering of a novel versatile fungal polyphenol oxidase with chlorophenol bioremediation potential**

**Authors: Efstratios Nikolaivits<sup>1</sup>, Maria Dimarogona<sup>2</sup>, Ioanna Karagiannaki<sup>1</sup>, Angelina Chalima<sup>1</sup>, Ayelet Fishman<sup>3</sup> and Evangelos Topakas<sup>1,4\*</sup>**

### **Affiliations:**

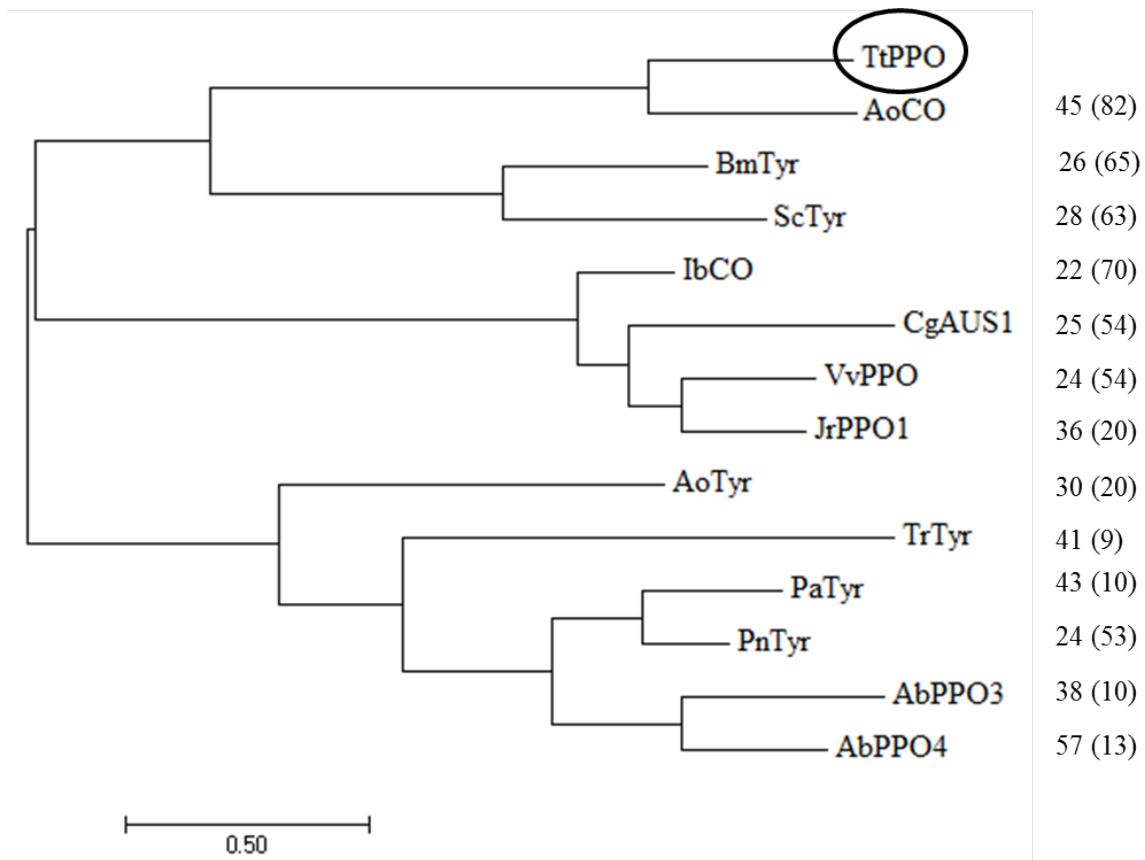
*<sup>1</sup>Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 15780 Athens, Greece*

*<sup>2</sup>Department of Chemical Engineering, University of Patras, 26504 Patras, Greece*

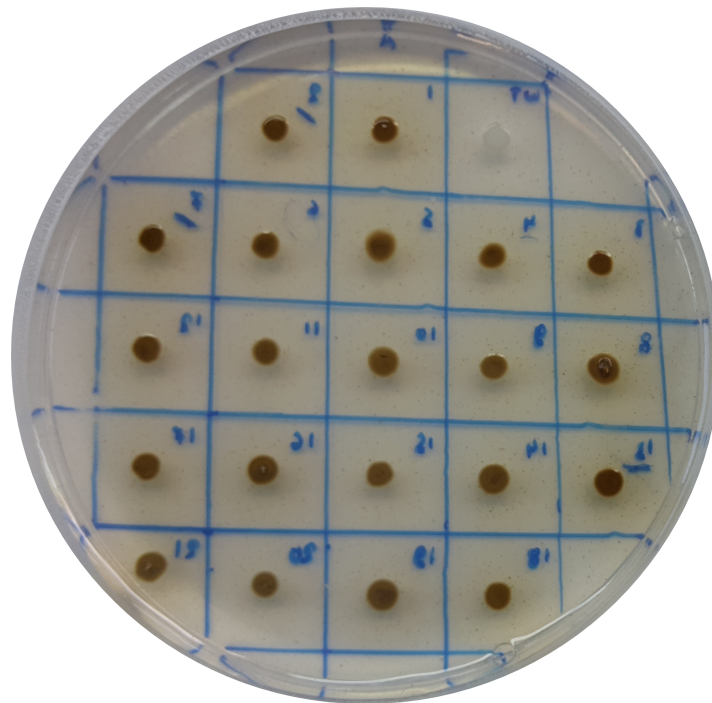
*<sup>3</sup>Department of Biotechnology and Food Engineering, Technion-Israel Institute of Technology, Haifa, 3200003, Israel*

*<sup>4</sup>Biochemical and Chemical Process Engineering, Division of Sustainable Process Engineering, Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, SE-97187 Luleå, Sweden*

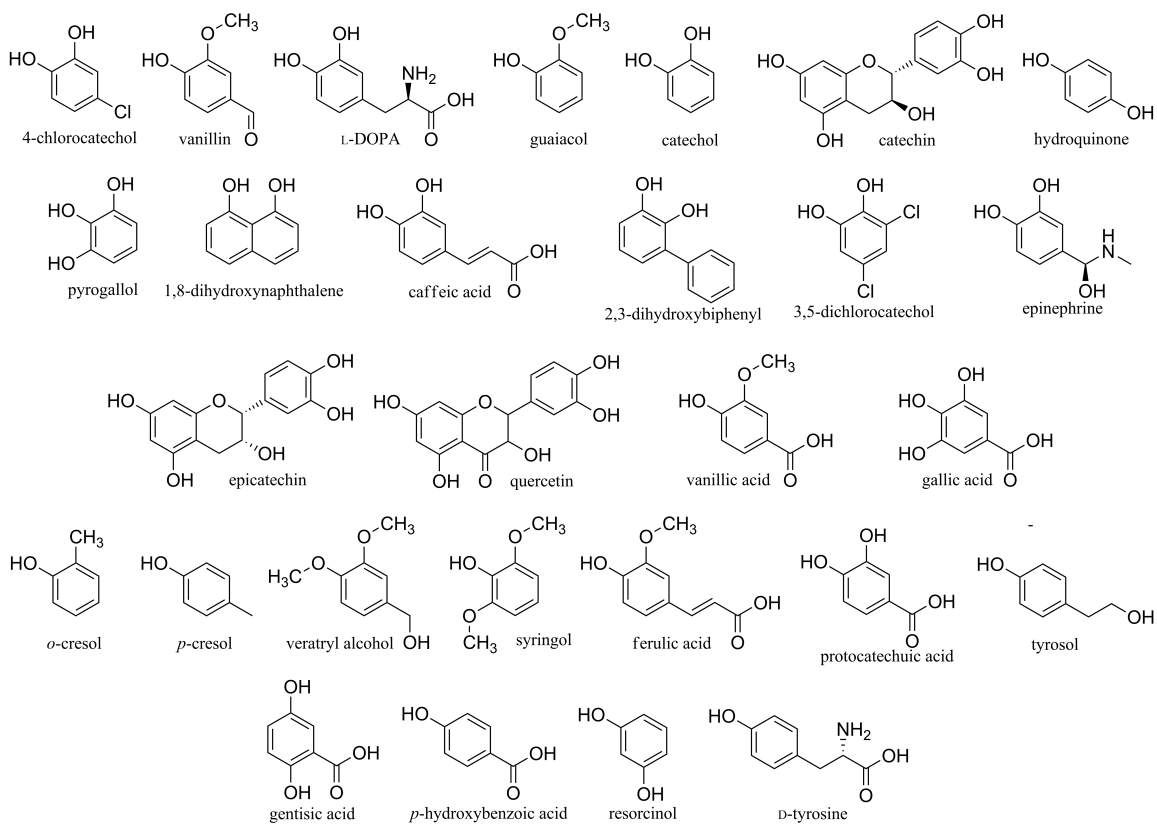
**\*Corresponding author's e-mail: [vtopakas@chemeng.ntua.gr](mailto:vtopakas@chemeng.ntua.gr)**



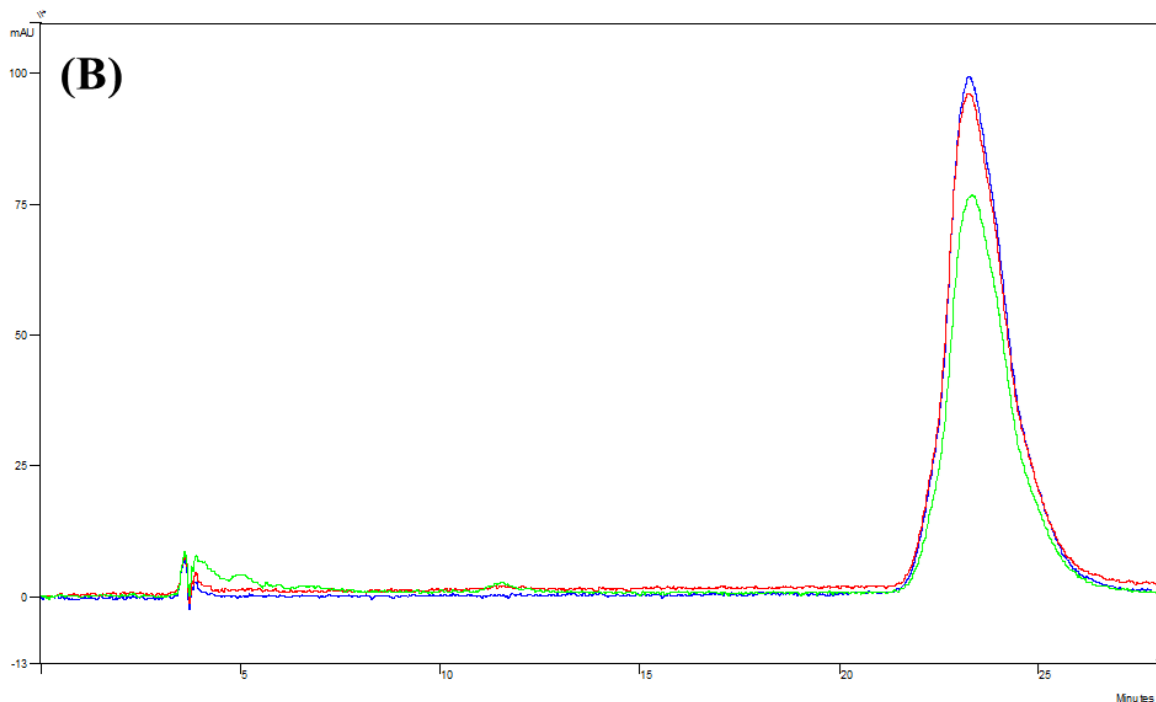
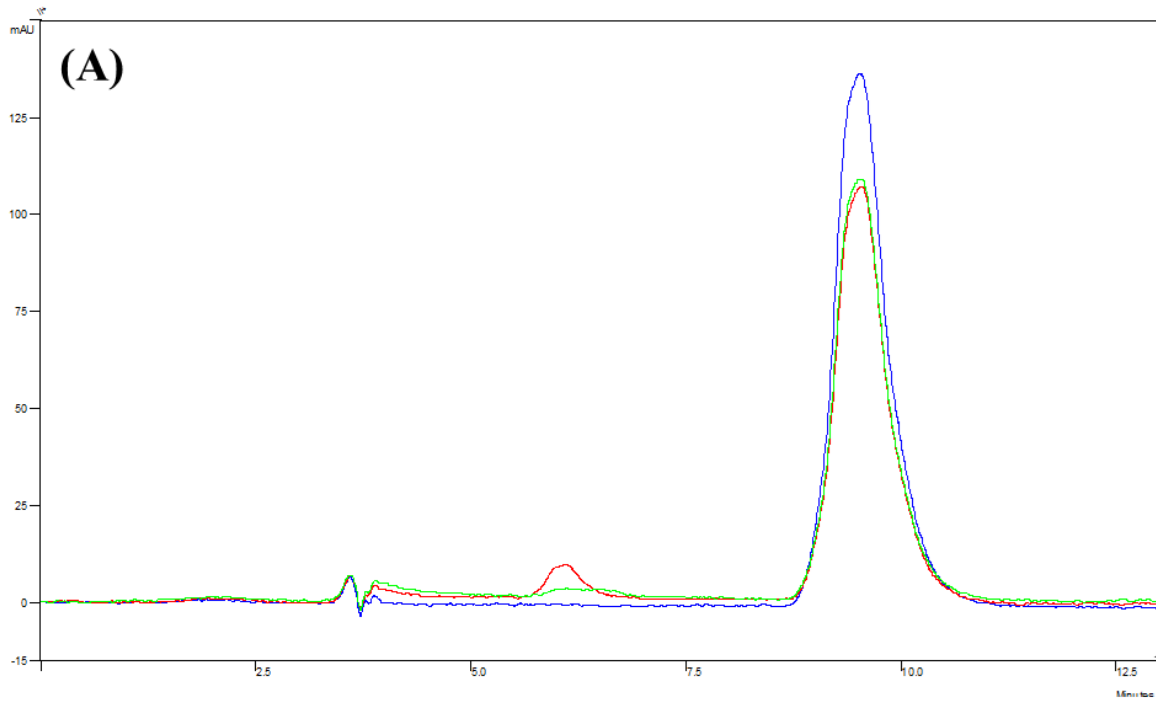
**Fig. S1** Phylogenetic tree based on known PPO sequences from different classes of organisms. Identity and query cover (in brackets) are presented as percentages (%) for each sequence according to the results of the BLAST compared to *TtPPO*. Abbreviations: PPO, polyphenol oxidase; CO, catechol oxidase; Tyr, tyrosinase; AUS, aurone synthase; Ao, *Aspergillus oryzae*; Bm, *Bacillus megaterium*; Sc, *Streptomyces castaneoglobisporus*; Ib, *Iponomea batatas*; Cg, *Coreopsis grandiflora*; Vv, *Vitis vinifera*; Jr, *Juglans regia*; Tr, *Trichoderma reesei*; Pa, *Polyporus arcularius*; Pn, *Pholiota nameko*; Ab, *Agaricus bisporus*.



**Fig S2.** Plate assay of Zeocin™ resistant transformants compared to the wild type *P. pastoris* X-33 strain (top right square) for detection of PPO activity.



**Fig. S3** Chemical structures of the compounds *Tt*PPO accepts as substrates, roughly placed according to the activity it presents on each of them.



**Fig. S4** HPLC chromatograms of (A) 3-CP reaction (blue: control, red: wild-type *TtPPO*, green: variant G292N/L306A) and (B) 3,5-diCP reaction (blue: control, red: wild-type *TtPPO*, green: variant G292N/Y296V)