Supporting information

(A)

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CtAPD       MQGEIIAGFLAPHPPHLVYGENPPQNEPRSQGGWEVLRWAYERARERLDAMKPDVLLVHS   60
PaDHPAO     -MGKVALAAKITHVPSLYLSELPGPRHGCRQPAIDGHR----EIGRRCRELGVDTIVVFD   55
KpDHPAO     -MGKLALAAKTTHVPSMYLSELPGKNHGCRQGAIDGHK----EIGKRCRELGVDTFIVFD   55
EcDHPAO     -MGKLALAAKITHVPSMYLSELPGKNHGCRQGAIDGHK----EISKRCREMGVDTIIVFD   55
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CtAPD       PHWITSVGHHFLGVPELSGKSVDPIFPN---VFRYDFSLNVDVELAEACAEEGRKAGLVT   117
PaDHPAO     THWLVNAGYHINCAPHFEGLYTSNELPHFIANMEYGFP--GNPELGRILAEGCNALGVET   113
KpDHPAO     THWLVNSAYHINCADHFQGVYTSNELPHFIRDMTYDYD--GNPELGHLIADKTVKLGVRA   113
EcDHPAO     THWLVNSAYHINCADHFEGVYTSNELPHFIRDMTYNYE--GNPELGQLIADEALKLGVRA   113
             **:.. .:*:  . .:.*  ..  :*:    : *.:    : **..  *:     *: :

CtAPD       KMMRNPKFRVDYGTITTLHLIRPQWDIPVVGISANNSPYYLNTKEGMSEMDVLGKATREA   177
PaDHPAO     LAHDATTLGPEYGTLVPMRYMNQDRHFKVVSVSALCTVHYLND------SARLGWAMRKA   167
KpDHPAO     KAHNIPSLKLDYGTLVPMRYMNADKHFKVVSISAFCTVHDFAD------SRKLGEAIRKA   167
EcDHPAO     KAHNIPSLKLEYGSVVPMRYMNEDKRFKVVSISAFCTVHDFAD------SRKLGERIVKA   167
                  .:  :**::. :: :. :  : **.:**  : : :           **    :*

CtAPD       IRK-TGRKAVLLASNTLSHWHFHEEPTIPEDMSKEYPATMAGYQWDIRMIELMRQGKTSE   236
PaDHPAO     VEEHYDGTVAFLASGSLSHRFAQ-NGQAPDFSDR-IWS-PFLEVLDHEVVQMWQEGRWAE   224
KpDHPAO     I-EKYDGTVAVLASGSLSHRFIE-DQRAEEGMNS-YTR-EFDHQMDERVVKLWREGKFKE   223
EcDHPAO     I-EQYDGTVAVLASGSLSHRFID-DQRAEEGMNS-YTR-EFDRQMDERVVKLWREGQFKE   223
            : :  . ....***.:*** . . :    :  .            * .:::: ::*:  *

CtAPD       VFKLLPQFIDEAFAEVKSGAFTWMHAAMQYPELAAELFGYGTVIGTGNAVMEWDLRKAG-   295
PaDHPAO     FCGMLPEYASKGHGEGFMHDTAML----------LGALGWSAYDGK-----AEVVTPYFG   269
KpDHPAO     FCTMLPENAEYCYGEGNMHDTVML----------LGLLGWDKYDGK-----EWNLSPNCL   268
EcDHPAO     FCNMLPEYADYCYGEGNMHDTVML----------LGMLGWDKYDGK-----VWSLSPSYS   268
            .  :**:  .  ..*      . :            :*:.  *.        :

CtAPD       ---LSMLGA------ADQKQRSAAVA----------        312
PaDHPAO     SSGTGQINAVFPVTAQDGSAIPAAQAGNPAGASCASRL      307
KpDHPAO     PASGTRPG-----------------------------       276
EcDHPAO     QASWHRSG-----------------------------       276
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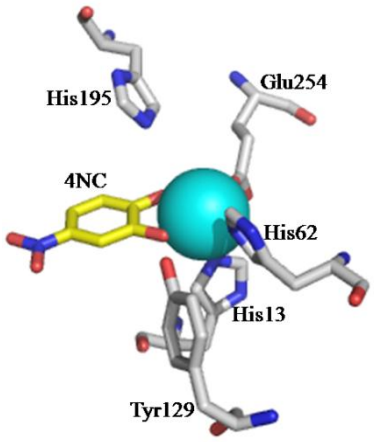
(B)



S1 Fig. A multiple sequence alignment of PaDHPAOs and other extradiol-ring cleavage dioxygenase in Cluster 1 and Cluster 6 (referred to Clusters in Fig. 2 main text). (A) The amino acid sequence of PaDHPAO was aligned with multiple sequences of *Klebsiella pneumoniae* (Kp) and *Escherichia coli* C (Ec) DHPAO enzymes in Cluster 1 and APD from *Comamonas testosteroni* (Ct) in Cluster 6 (A). Three amino acid residues (highlighted in yellow color) of PaDHPAO (His12, His57, and Glu239) and other DHPAOs in Cluster 1 are well conserved when compared to the CtAPD active-site residues (His13, His62, and Glu251). The other two active-site residues (highlighted in cyan, Tyr129 and His195) are also conserved in PaDHPAO (Tyr125 and His186) and other DHPAOs in Cluster 1. These two residues are important for second sphere metal-coordination and were shown to be important for catalysis [42]. (B) The active site structure of CtAPD co-crystallized with 4-nitrocatechol (4NC) shows the involvement of all these conserved residues in metal coordination and substrate binding. As these residues are conserved in PaDHPAO, it is likely that the enzyme is a member of the 2-His-1-carboxylate enzyme superfamily similar to CtAPD.