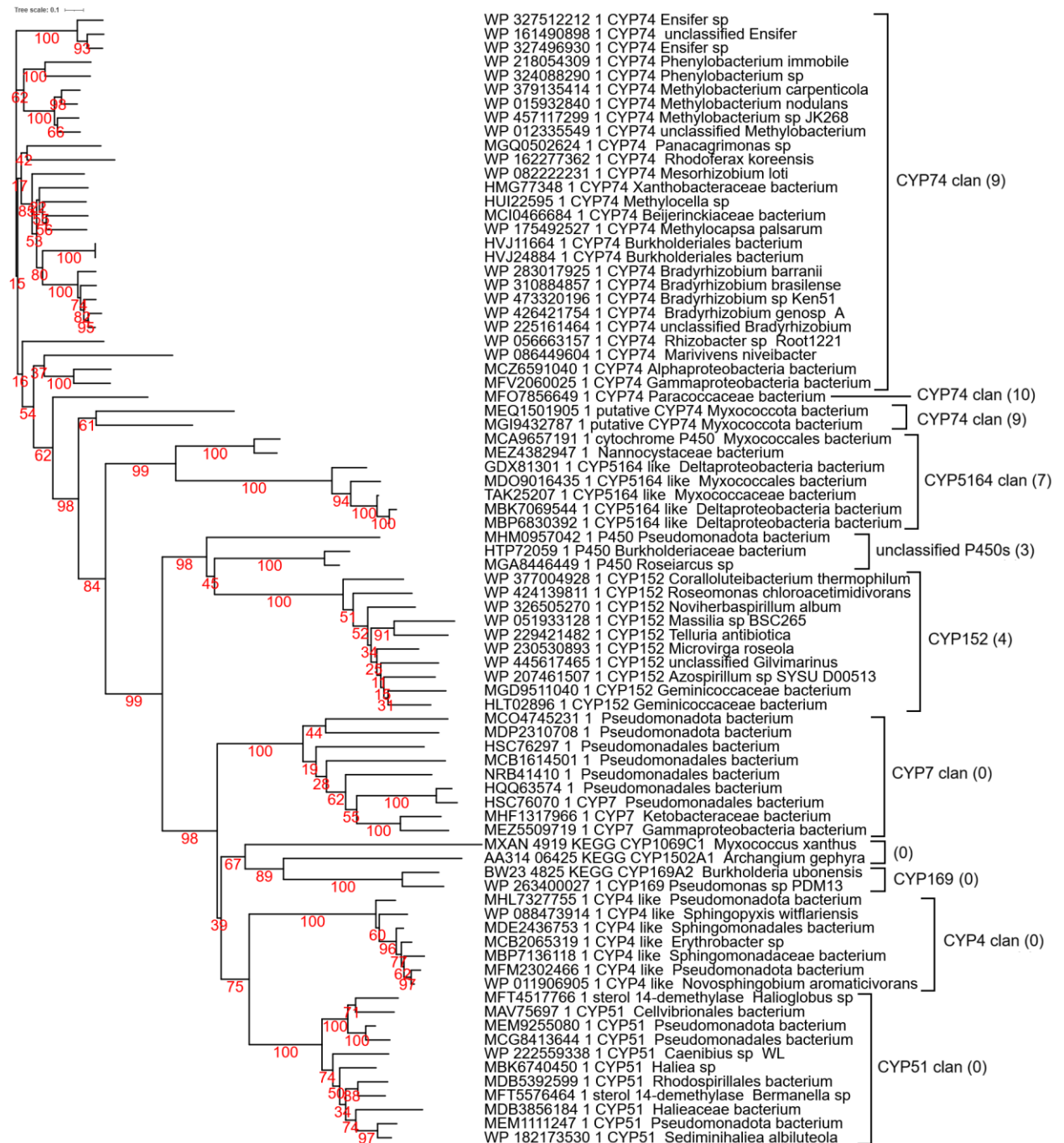


Supplementary Information

for the article

Phylogenetic and structural studies uncover the ancestral role of CYP74 clan proteins among proteobacterial P450s

by Alexander N. Grechkin



Supplementary Figure S1. Phylogenetic tree of selected proteobacterial P450s. A multiple sequence alignment of 81 proteobacterial P450 proteins was constructed using the MAFFT version 7 web-based platform (Katoh et al., 2019) with the L-INS-i strategy. Gappy regions of the alignment were automatically trimmed by MAFFT software prior the construction of the tree. The phylogenetic tree was constructed using the MAFFT software and the Neighbor-Joining (NJ) method (JTT model; Bootstrap resampling = 100). The tree was visualized with iTOL online software. The red numbers below the branches represent the bootstrap values.

Supplementary Discussion

The CYP51 clan proteins are often considered the ancestors of the most eukaryotic P450s (Yoshida et al., 2000; Nelson and Nebert, 2018). The CYP51s, certainly, played the ancestral role for a large diversity of eukaryotic P450 monooxygenases. However, the CYP51s were evolved very early in Proteobacteria. Furthermore, they were not the ancestral P450s in Proteobacteria. A substantial diversity of P450s emerged from ancestral proteins in Proteobacteria before eukaryogenesis, as seen from Nzuza et al. (2021), Msomi et al. (2021), and Msweli et al. (2022), as well as from the results of this work. Among the P450s from the Proteobacteria phylum, CYP51s are located at the top of the phylogenetic tree. As noted by McLean et al. (2015), “although CYP51 is likely an early P450, it is not the progenitor in the P450 gene superfamily.” As proposed previously, the hypothetical P450 progenitor was involved in metabolism that was not dependent on molecular oxygen and redox partners (Kahn and Durst, 2000; Werck-Reichhart and Feyereisen, 2000; McLean et al., 2015).

As mentioned in the main text of this article, many proteobacterial CYP74s are fusion proteins. The CYP74 clan domain from the *Methylobacterium nodulans* fusion protein has been cloned and identified as the hydroperoxide lyase (Lee et al., 2008). The full-length DNA has been prepared by PCR amplification and sequenced to confirm the structure (Lee et al., 2008). However, the peroxidase (cyclooxygenase-like) domain has not been cloned, and the recombinant proteins have not been studied. The *M. nodulans* hydroperoxide lyase (Lee et al., 2008) remains the only proteobacterial CYP74 cloned and characterized to date. Nevertheless, the results obtained with the *M. nodulans* enzyme, as well as its fusion structure (including the peroxidase presumably functioning as a dioxygenase), indicate the probable cooperative role of two domains in oxylipin biosynthesis. Thus, most likely, the proteobacterial CYP74s are involved in oxylipin biosynthesis in Proteobacteria.

Supplementary Reference

Nelson DR, Nebert DW (2018) Cytochrome P450 (CYP) gene superfamily. In: eLS. John Wiley & Sons Ltd, Chichester, pp 1-19. <https://doi.org/10.1002/9780470015902.a0005667.pub3>

The FASTA dataset used to generate the multiple alignments and construct the phylogenetic trees

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>WP_327496930.1_CYP74_Ensifer_sp

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>WP_327512212.1_CYP74_Ensifer_sp

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>HVJ24884.1_CYP74_Burkholderiales_bacterium

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>HVJ11664.1_CYP74_Burkholderiales_bacterium

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>WP_175492527.1_CYP74_Methylocapsa_palsarum

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>WP_161490898.1 CYP74 [unclassified Ensifer]

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>MGQ0502624.1 CYP74 [Panacagrimonas sp.]

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>WP_086449604.1 CYP74 [Marivivens niveibacter]

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>WP_426421754.1 CYP74 [Bradyrhizobium genosp. A]

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>WP_162277362.1 CYP74 [Rhodoferax koreensis]

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>WP_056663157.1 CYP74 [Rhizobacter sp. Root1221]

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>MFT5576464.1 sterol 14-demethylase [Bermanella sp.]
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>MDB5392599.1 CYP51 [Rhodospirillales bacterium]
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>MBK6740450.1 CYP51 [Haliea sp.]
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>WP_182173530.1 CYP51 [Sediminihaliea albiluteola]
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>MEM1111247.1 CYP51 [Pseudomonadota bacterium]
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>MCG8413644.1 CYP51 [Pseudomonadales bacterium]
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>MAV75697.1 CYP51 [Cellvibrionales bacterium]
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>WP_011906905.1 CYP4_like [Novosphingobium aromaticivorans]
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>MDE2436753.1 CYP4_like [Sphingomonadales bacterium]
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>MCB2065319.1 CYP4_like [Erythrobacter sp.]
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PAHA
>MBP7136118.1 CYP4_like [Sphingomonadaceae bacterium]
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>WP_088473914.1 CYP4_like [Sphingopyxis witflariensis]
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