

Electronic Supplementary Information

**Enzymatic Synthesis of ω -hydroxydodecanoic acid employing a
Cytochrome P450 from *Limnobacter* sp. 105 MED**

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Table S1**Plasmids and strains used in this study**

Plasmids/Strains	Description	Reference
Plasmids		
pET24ma	P15A ori lacI T7 promoter, KmR	[1]
pETDuet-1	pBR322 ori lacI T7 promoter, AmpR	<i>Novagen</i>
pCDFDuet-1 CDF	CDF ori lacI T7 promoter, SmR	<i>Novagen</i>
pCamAB	pETDuet-1 encoding CamA/B	[2]
pRedox _{L,m}	pETDuet-1 encoding LimA/B	This study
pAM.aq	pCDFDuet-1 CDF encoding fadL and CYP153AM.aq	This study
pAA.d	pCDFDuet-1 CDF encoding fadL and CYP153AA.d	This study
pAS.f	pCDFDuet-1 CDF encoding fadL and CYP153AS.f	This study
pAL.m	pCDFDuet-1 CDF encoding fadL and CYP153AL.m	This study
pCYP153A _{M,aq}	pET28a encoding CYP153A _{M,aq}	[1]
pCamA	pET28a encoding CamA	[1]
pCamB	pET28a encoding CamB	[1]
pCYP153A _{L,m}	pET24ma encoding CYP153A _{L,m}	This study
pLimA	pet24ma encoding LimA	This study
pLimB	pet24ma encoding LimB	This study

Strains		
BW25113(DE3)	rrnB3 ΔlacZ4787 hsdR514 Δ(araBAD)567 Δ(rhaBAD)568 [2] rph-1 λ(DE3)	
DL	BW25113(DE3) ΔfadD	[2]
MC	DL carrying pAM.aq and pCamAB	This study
DC	DL carrying pAA.d and pCamAB	This study
SC	DL carrying pAS.f and pCamAB	This study
LC	DL carrying pCYP153AL.m and pCamAB	This study
LL	DL carrying pCYP153AL.m and pRedox _{L.m}	This study
DLM	DL carrying pCYP153 M.aq	This study
DLCA	DL carrying pCamA	This study
DLCB	DL carrying pCamB	This study
DLL	DL carrying pCYP153L.m	This study
DLLA	DL carrying pLimA	This study
DLLB	DL carrying pLimB	This study

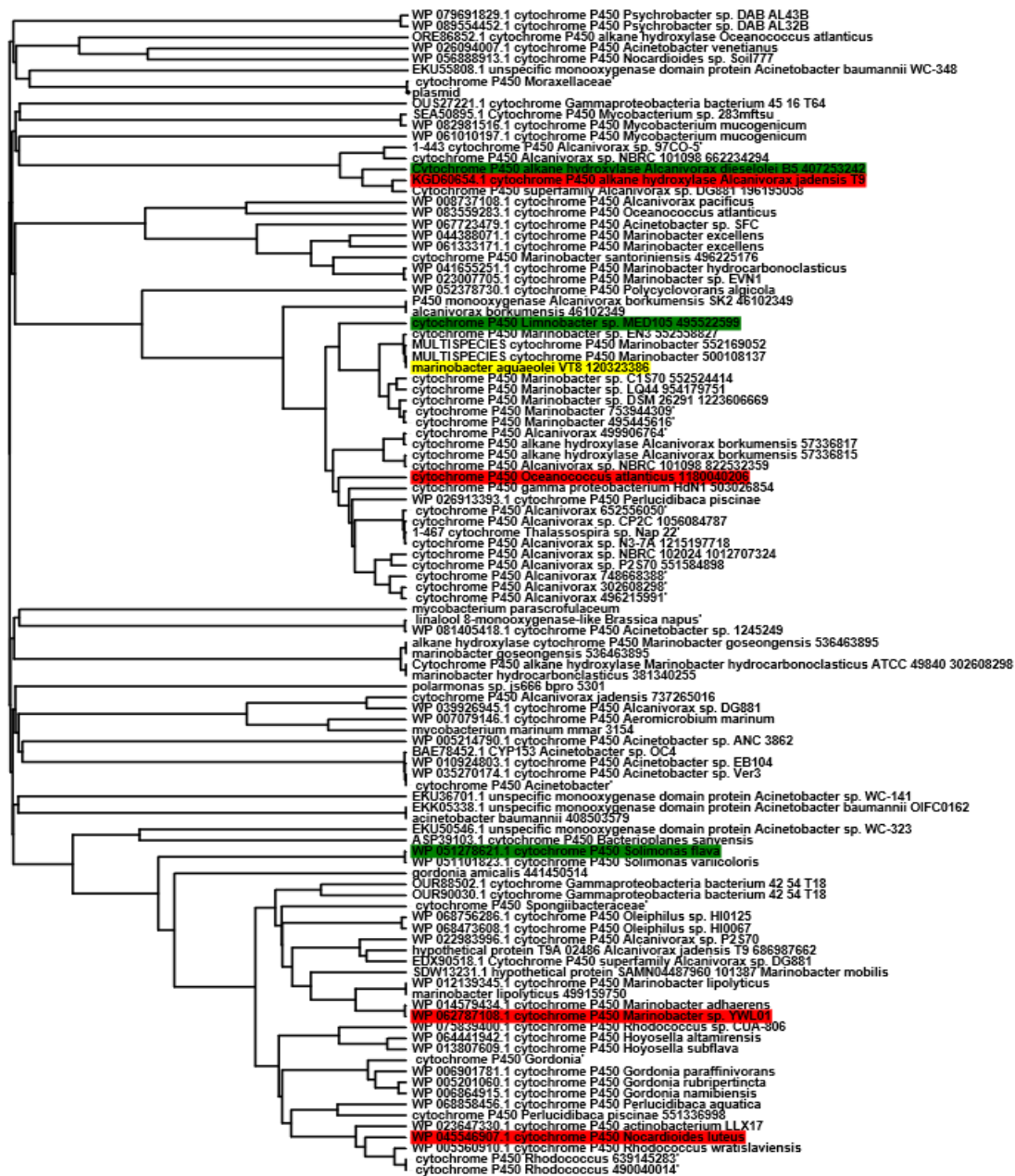


Figure S1. Phylogenetic tree used in this study

The phylogenetic tree was constructed through Maximum like hood tree algorithm in

software MEGA7. Initially, 100 candidates were obtained by Blastp

[<http://blast.ncbi.nlm.nih.gov>], using maqu_0600 sequence. Yellow color: CYP153AM.aq,

green color: expressed P450s and Red color: not expressed P450.

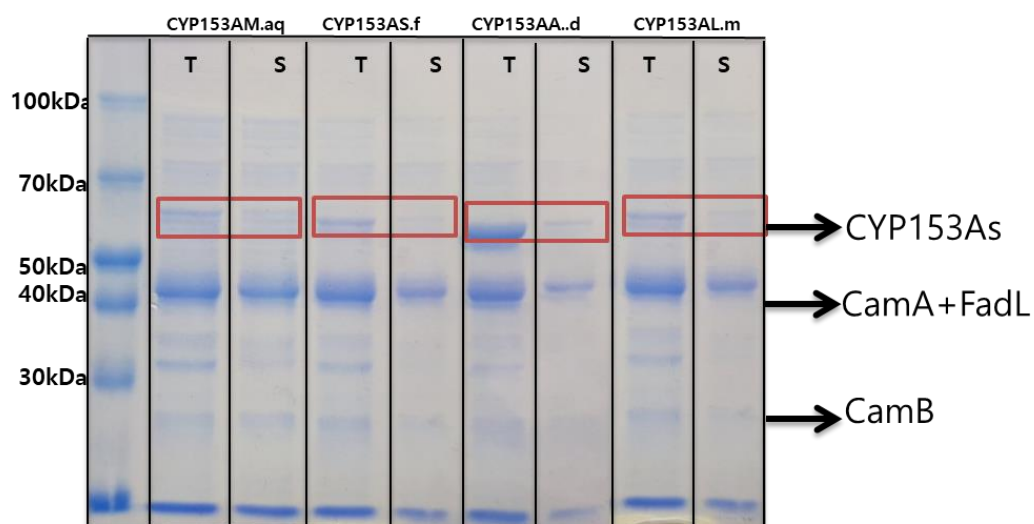


Figure S2. A SDS-PAGE analysis of protein expression of CYP153As

E. coli BW25113 ($\Delta fadD$, DE3) was used, Protein expression was carried out using 0.01 mM IPTG, 0.5 mM 5-ALA and trace mineral mixtures (2.5 mL/L) at 30 °C temp. CamB (12.75 kDa), CamA (47 kDa), FadL(48.8kDa), CYP153As (52.28 kDa)

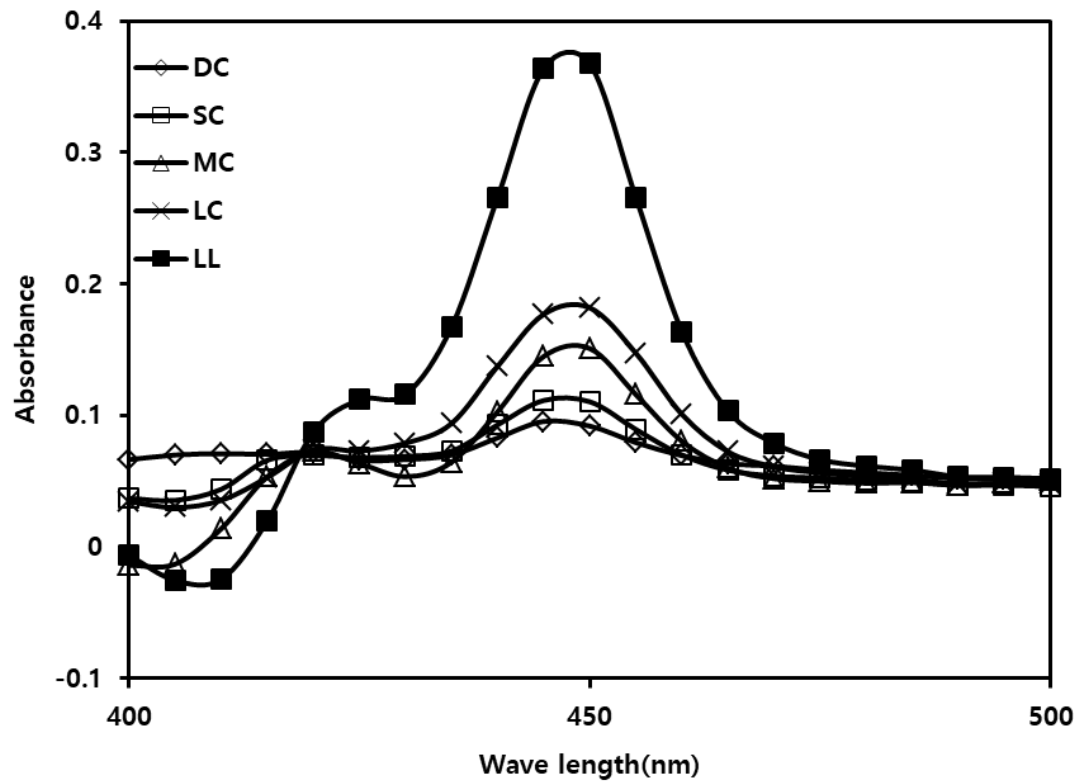


Figure S3. A CO-binding analysis of CYP153A expressing strains used in this study

MC= *E. coli* BW25113 (Δ *fadD*, DE3) having CYP153AM.aq+CamA+CamB+FadL

DC= *E. coli* BW25113 (Δ *fadD*, DE3) having CYP153AA.d+CamA+CamB+FadL

SC= *E. coli* BW25113 (Δ *fadD*, DE3) having CYP153AS.f +CamA+CamB+FadL

LC= *E. coli* BW25113 (Δ *fadD*, DE3) having CYP153AL.m+ CamA+CamB+FadL

LL= *E. coli* BW25113 (Δ *fadD*, DE3) having CYP153AL.m+LimA+LimB+FadL

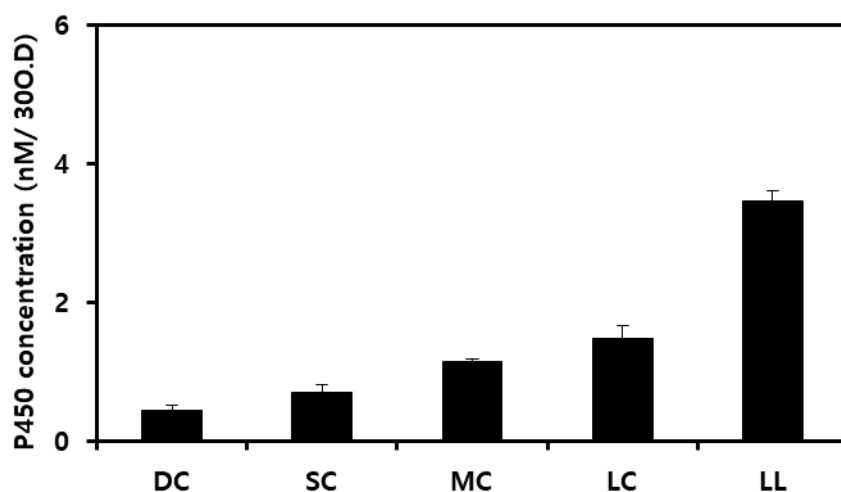


Figure S4. An active P450 concentration used in this study.

The concentration of P450 was measured using an extinction coefficient of $91.9 \text{ mM}^{-1} \text{ cm}^{-1}$ at 450 nm.

MC= *E. coli* BW25113 ($\Delta fadD$, DE3) having CYP153AM.aq+CamA+CamB+FadL

DC= *E. coli* BW25113 ($\Delta fadD$, DE3) having CYP153AA.d+CamA+CamB+FadL

SC= *E. coli* BW25113 ($\Delta fadD$, DE3) having CYP153AS.f +CamA+CamB+FadL

LC= *E. coli* BW25113 ($\Delta fadD$, DE3) having CYP153AL.m+ CamA+CamB+FadL

LL= *E. coli* BW25113 ($\Delta fadD$, DE3) having CYP153AL.m+LimA+LimB+FadL

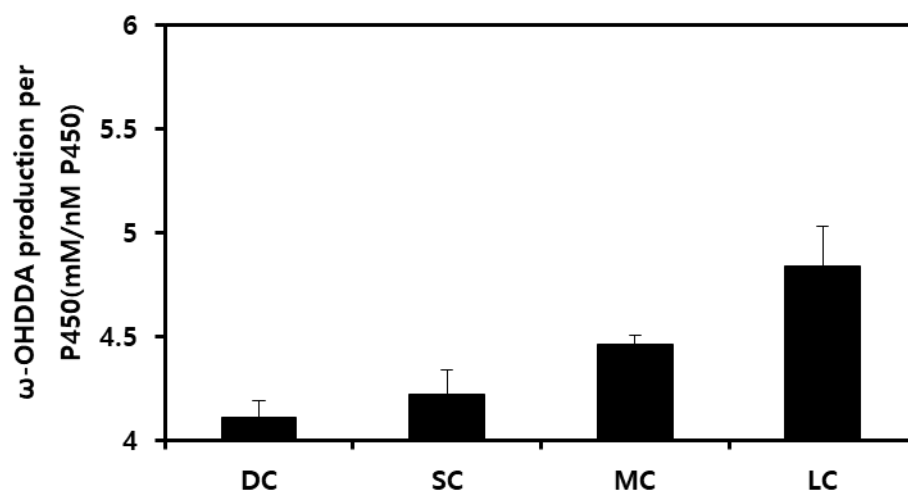


Figure S5. ω -OHDDA production normalized by amount of active P450s.

(The final titer in Figure.2 was normalized by the amount of active P450s in Figure S3)

MC= *E. coli* BW25113 ($\Delta fadD$, DE3) having CYP153AM.aq+CamA+CamB+FadL

DC= *E. coli* BW25113 ($\Delta fadD$, DE3) having CYP153AA.d+CamA+CamB+FadL

SC= *E. coli* BW25113 ($\Delta fadD$, DE3) having CYP153AS.f +CamA+CamB+FadL

LC= *E. coli* BW25113 ($\Delta fadD$, DE3) having CYP153AL.m+ CamA+CamB+FadL

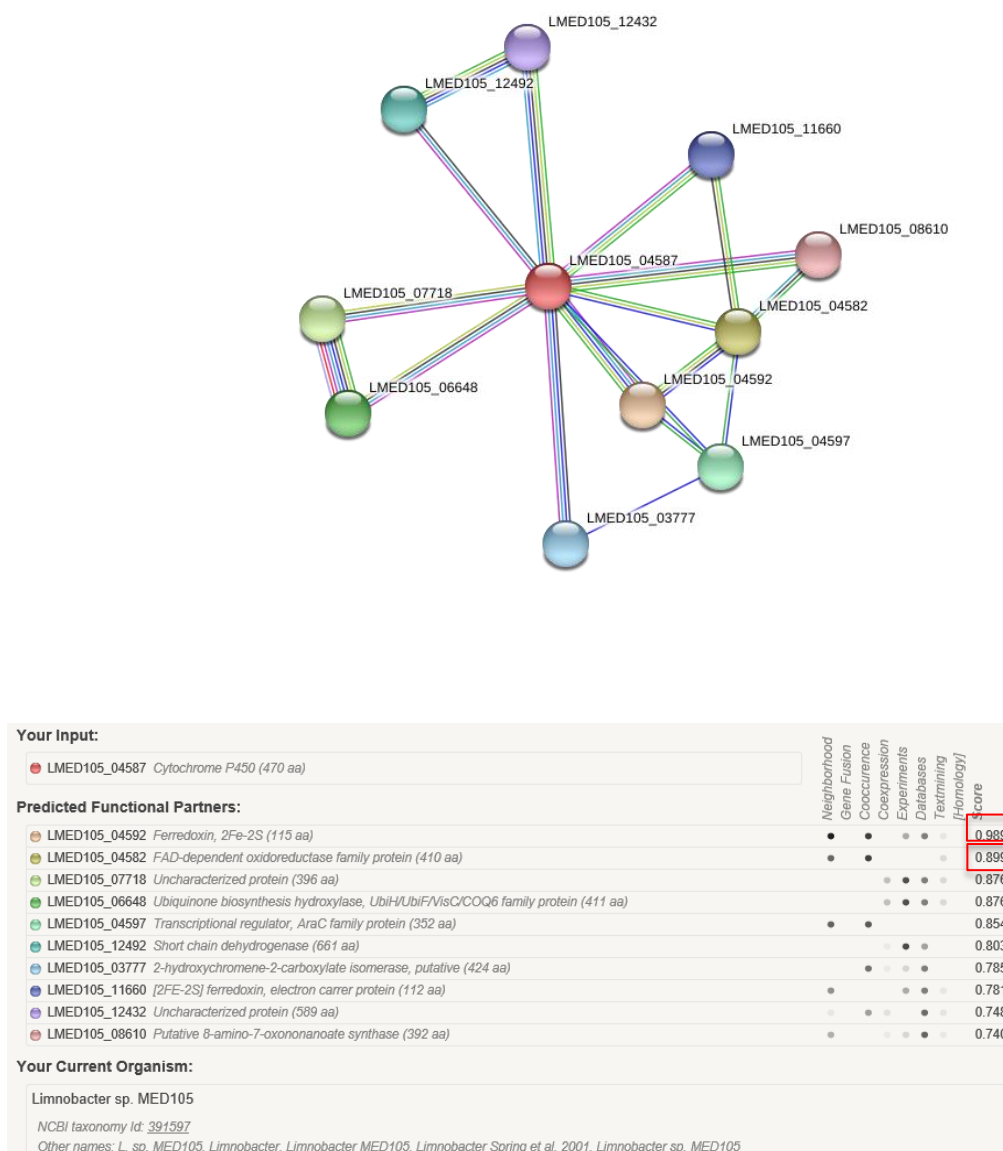


Figure S6. Protein-protein network of CYP153AL.m(LMED105_04587).

There are two 2Fe-2S ferredoxin in the network (LMED105_04592, LMED105_11660) having score 0.989 and 0.781 respectively. LMED105_04592(LimB) was used in this study as it has higher score than LMED105_11660 also LMED105_04582(LimA) was used as its corresponding reductase.

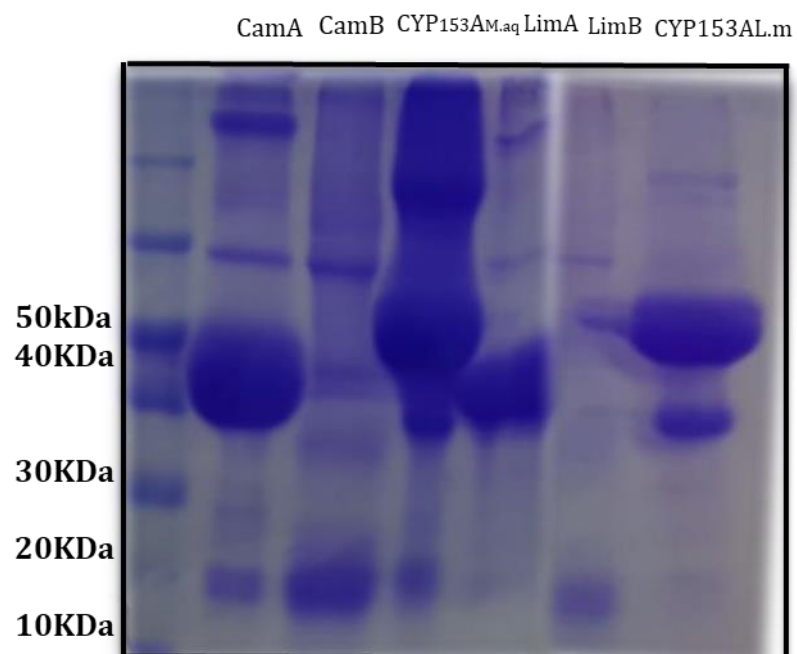


Figure S7. SDS-PAGE gel picture of purified protein of CamB (12.75 kDa), CamA (47 kDa), CYP153AM.aq (52.28 kDa) ,LimB (11.87 kDa), LimA (45.61 kDa), and CYP153AL.m (52.28 kDa).

Reference.

- [1] Jung, E., Park, B. G., Ahsan, M. M., Kim, J., et al., Production of ω -hydroxy palmitic acid using CYP153A35 and comparison of cytochrome P450 electron transfer system in vivo. *Applied microbiology and biotechnology* 2016, 100, 10375-10384.
- [2] Bae, J. H., Park, B. G., Jung, E., Lee, P.-G., Kim, B.-G., fadD deletion and fadL overexpression in *Escherichia coli* increase hydroxy long-chain fatty acid productivity. *Applied microbiology and biotechnology* 2014, 98, 8917-8925.
- [3] Szklarczyk, D., Morris, J. H., Cook, H., Kuhn, M., *et al.*, The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic acids research* 2017, 45, D362-D368.