

**Electronic Supplementary Information**

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

Sung-yeon ju<sup>1,2\*</sup>

Hee-Wang Yoo<sup>1,2\*</sup>

Sharad Sarak<sup>1</sup>

Byung-Gee Kim<sup>2</sup>

Hyungdon Yun<sup>1</sup>

<sup>1</sup>Department of Systems Biotechnology, Konkuk University, Seoul, South Korea

<sup>2</sup>School of Chemical and Biological Engineering, Seoul National University, Seoul,  
South Korea

\*These authors contributed equally

**Correspondence:** Prof. Yun Hyungdon, Department of Systems Biotechnology,  
Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul-050-29, South Korea

**E-mail:** [hyungdon@konkuk.ac.kr](mailto:hyungdon@konkuk.ac.kr)

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

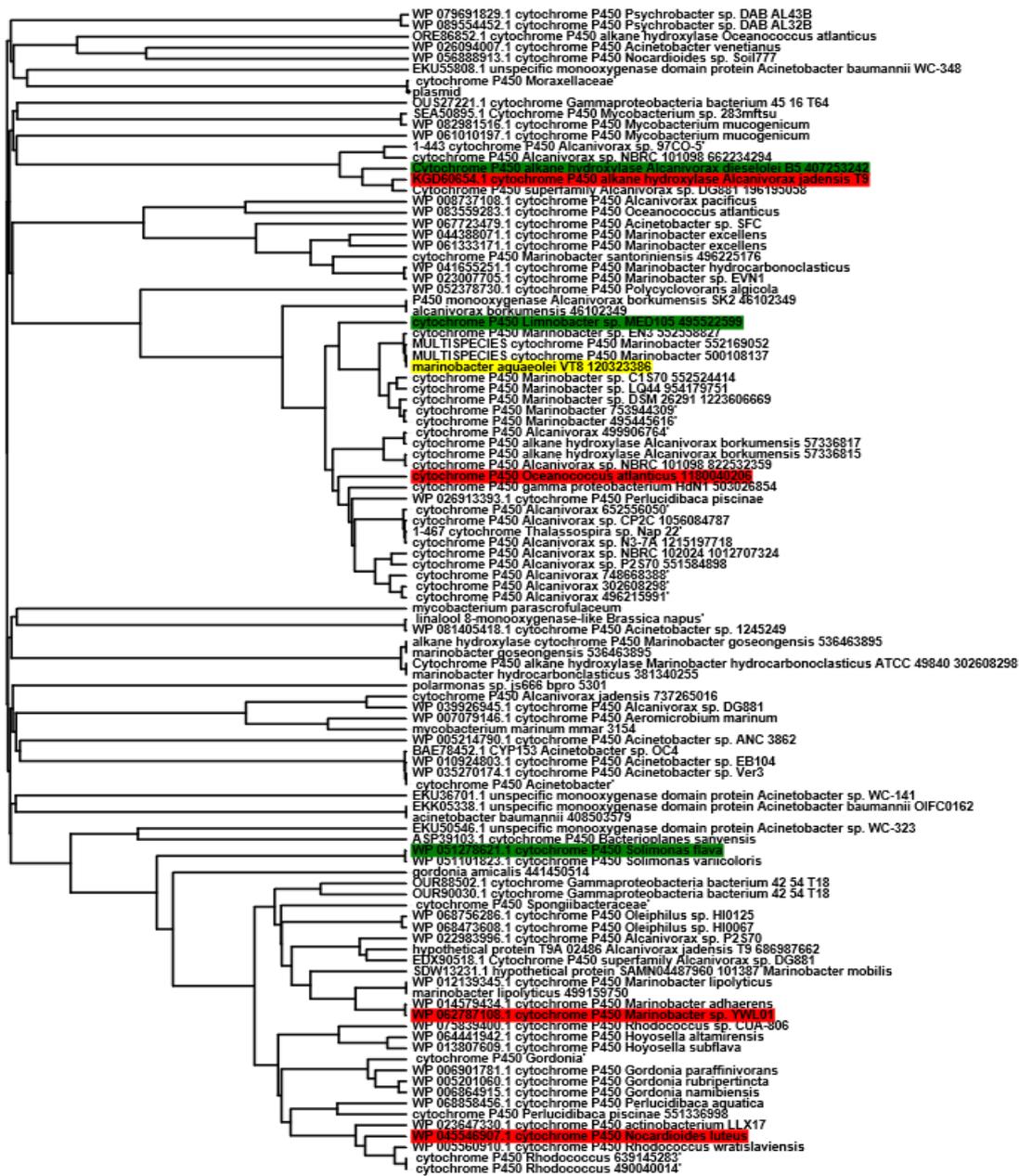
<b>Plasmids/Strains</b>	<b>Description</b>	<b>Reference</b>
<b>Plasmids</b>		
pET24ma	P15A ori lacI T7 promoter, KmR	[1]
pETDuet-1	pBR322 ori lacI T7 promoter, AmpR	<i>Novagen</i>
pCDFDuet-1 CDF	CDF ori lacI T7 promoter, SmR	<i>Novagen</i>
pCamAB	pETDuet-1 encoding CamA/B	[2]
pRedox <sub>L,m</sub>	pETDuet-1 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 <sub>M,aq</sub>	pET28a encoding CYP153A <sub>M,aq</sub>	[1]
pCamA	pET28a encoding CamA	[1]
pCamB	pET28a encoding CamB	[1]
pCYP153A <sub>L,m</sub>	pET24ma encoding CYP153A <sub>L,m</sub>	This study
pLimA	pet24ma encoding LimA	This study
pLimB	pet24ma encoding LimB	This study

---

**Strains**

BW25113(DE3)	rrnB3 $\Delta$ lacZ4787 hsdR514 $\Delta$ (araBAD)567 $\Delta$ (rhaBAD)568 [2] rph-1 $\lambda$ (DE3)	
DL	BW25113(DE3) $\Delta$ fadD	[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 <sub>L,m</sub>	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

---



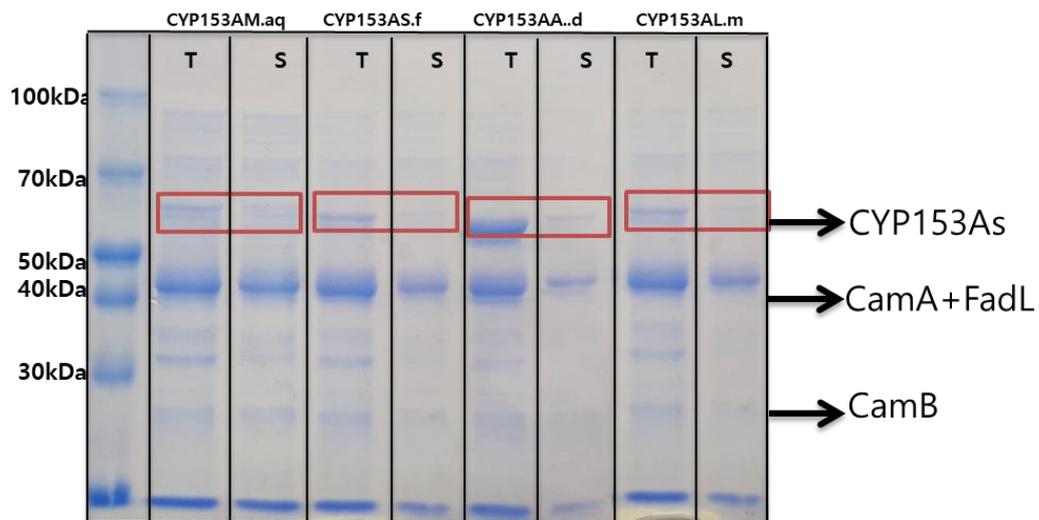
**Figure S1. Phylogenetic tree used in this study**

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,

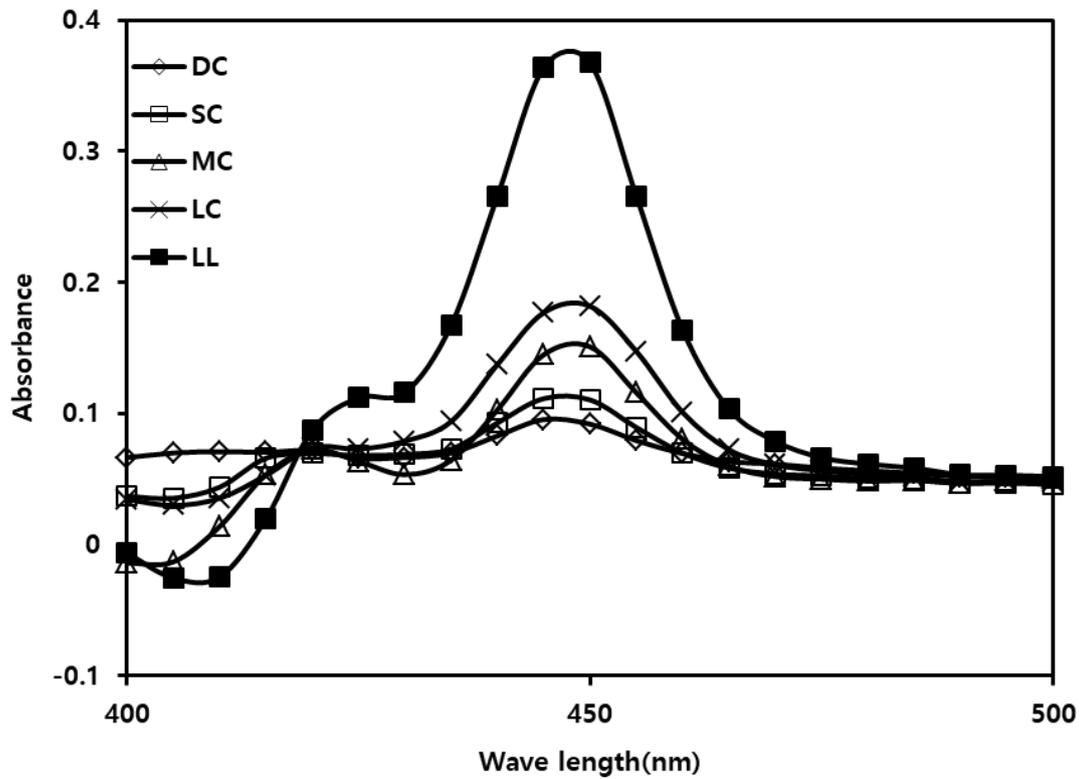
green color: expressed P450s and Red color: not expressed P450.





**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**

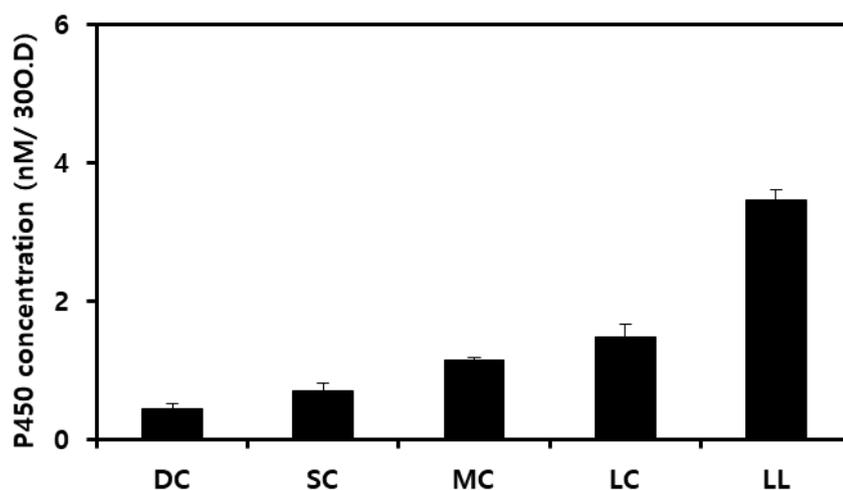
MC= *E. coli* BW25113 ( $\Delta$ *fadD*, DE3) having CYP153AM.aq+CamA+CamB+FadL

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 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.

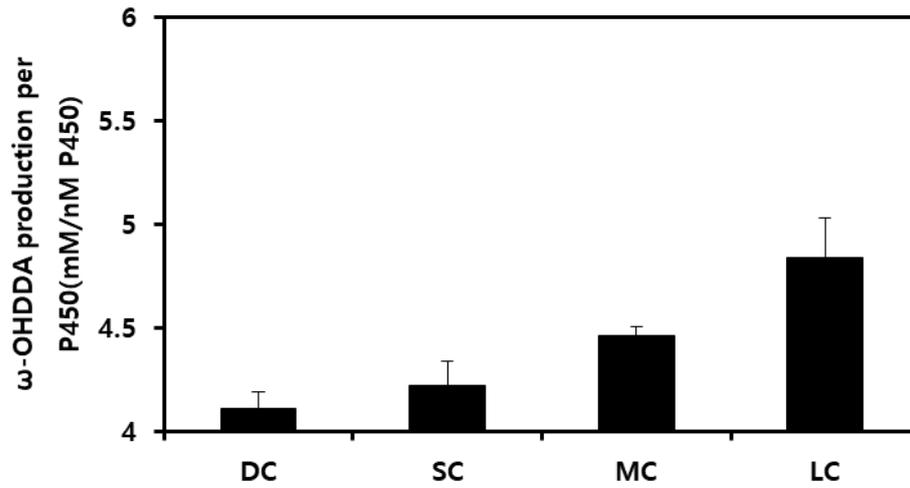
MC= *E. coli* BW25113 ( $\Delta fadD$ , DE3) having CYP153AM.aq+CamA+CamB+FadL

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.  $\omega$ -OHDDA production normalized by amount of active P450s.**

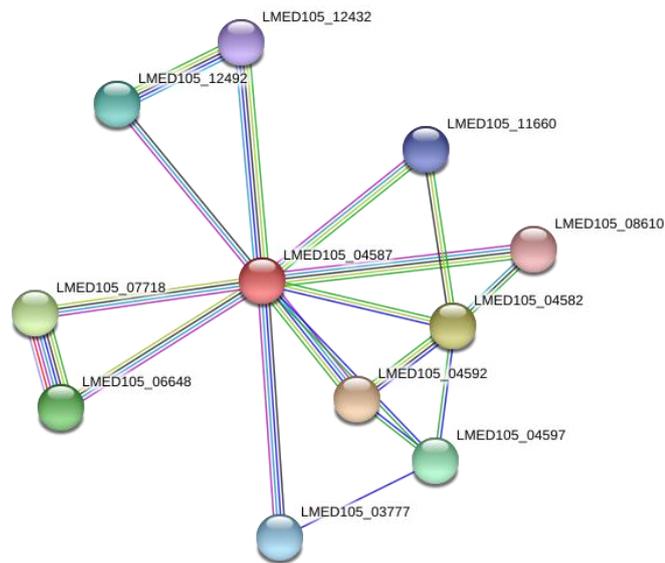
(The final titer in Figure.2 was normalized by the amount of active P450s in Figure S3)

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



**Your Input:**

● LMED105\_04587 Cytochrome P450 (470 aa)

**Predicted Functional Partners:**

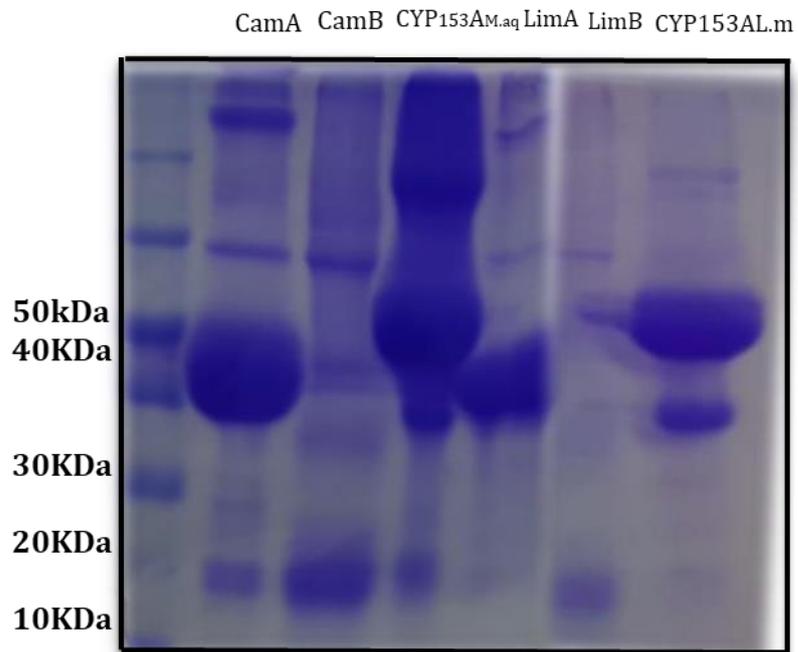
	Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	Homology	Score
● LMED105_04592 Ferredoxin, 2Fe-2S (115 aa)	●								0.989
● LMED105_04582 FAD-dependent oxidoreductase family protein (410 aa)	●	●							0.899
● LMED105_07718 Uncharacterized protein (396 aa)				●	●	●	●		0.876
● LMED105_06648 Ubiquinone biosynthesis hydroxylase, UbiH/UbiF/VisC/COQ6 family protein (411 aa)				●	●	●	●		0.876
● LMED105_04597 Transcriptional regulator, AraC family protein (352 aa)		●	●						0.854
● LMED105_12492 Short chain dehydrogenase (661 aa)					●	●	●		0.803
● LMED105_03777 2-hydroxychromene-2-carboxylate isomerase, putative (424 aa)				●	●	●	●		0.785
● LMED105_11660 [2Fe-2S] ferredoxin, electron carrier protein (112 aa)				●	●	●	●		0.781
● LMED105_12432 Uncharacterized protein (589 aa)				●	●	●	●		0.748
● LMED105_08610 Putative 6-amino-7-oxononanoate synthase (392 aa)		●		●	●	●	●		0.740

**Your Current Organism:**

Limnobacter sp. MED105  
 NCBI taxonomy id: 391597  
 Other names: L. sp. MED105, Limnobacter, Limnobacter MED105, Limnobacter Spring et al. 2001, Limnobacter sp. MED105

**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  $\omega$ -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.