

SUPPLEMENTARY DATA

for

Fatty acid biosynthesis in *Pseudomonas aeruginosa* is initiated by FabY: A new class of β -ketoacyl acyl carrier protein synthases

Yanqiu Yuan[†], Meena Sachdeva[†], Jennifer A. Leeds[†], and Timothy C. Meredith[†]
[†] Infectious Diseases Area, Novartis Institutes for BioMedical Research, Cambridge MA 02139, USA.

Table S1: List of Primers

Primers	Sequence
fabH Se for EcoRI ^a	aaaaaagaattcgtatataaccgaaaagtgactg
fabH Se rev KpnI ^a	aaaaaaggtagccacaaatgcaaattgcgtcat
gutAEB pKD4 P1	atggtgcagagtggtttatcgggctgttccaaaagggcggagaggtgtttaagggcgacacggaatgtg
gutAEB pKD4 P2	cgatcccggcgcgatatcgtcggggacagggcctgccacatggacagtgcccaagccagggatgtaacgc
fabH Ec KO P1	cgccacattgccgcgccaacgaaaccgtttcaacctgggccgctacctgtgacggaa
fabH Ec KO P2	ccgcccagatttcagttatgatcggtctacgttaatgcatgaactcatttaaatggcgcg
pWebUp Seq	cctgacgtctaagaaacct
pWebDown Seq	acacgggtgctgactgcg
PA3333 P1 ^b	tacaaaaaagcaggtccacctgctgatgatgagc
PA3333 P2 ^b	cccatccactaaattfaaafagctgccagggccgcagac
PA3333 P3 ^b	tattaaatttagtgatgggacgttccgacaatggactg
PA3333 P4 ^b	tacaagaaagctgggtgaatacagagcgcgagtagaag
PA0999 P1 BamHI ^a	cttagaggatccgagaacgagttcag
PA0999 P2 ^a	gtaggtcaggaccagcagctattgatgcg
PA0999 P3 ^a	cgcataatacgtcgtggtctgacctac
PA0999 P4 KpnI ^a	cttagtggtaccgtctcagtcacagag
PA0998 P1 BamHI ^a	cttagaggatccatctcggcaacagattc
PA0998 P2 ^a	ggcatcaggggtgtagctgaactcgttctc
PA0998 P3 ^a	gagaacgagttcagctacacctgatgacc
PA0998 P4 EcoRI ^a	gcttatgaattcagttcgtcagggattc
PA3286 P1 BamHI ^a	cttagaggatcctcagtcgggttttc
PA3286 P2 ^a	catgttcaggttgccctggctgttctgactg
PA3286 P3 ^a	cagtacaacgaccaggccaacctgaacatg
PA3286 P4 KpnI ^a	cttagtggtaccgtagggcgcatactg
PA5174 only for ^c	tagggagaggatcccgtccgtgaagctcgc
PA5174 only rev ^c	gtcagcagggatcccgccttttccgttgtg
pET24-PA5174 for ^c	aaggagatatacatatgtctcgactaccggctat
pET24-PA5174 rev ^c	gtgcggccgcaagcttgcgagcatgtcgcctgaaa
PA5174 P1 ^c	ggccagtgccaagctcagaacccgaccaag
PA5174 P2 ^c	gaaaccaacaatgacc
Gent for PA5174 ^c	gtcattgttggttccgagctcgaattgac
Gent rev PA5174 ^c	ggttatataccgtcaggcttgaacgaattg
fabH Ec for ^c	tgacggtatataaccg
fabH Ec rev ^c	gcaaattgcgtcatgt
PA5174 P3 ^c	catgacgcaattgccatctacaactcgg
PA5174 P4 ^c	acgaattcgagctcagtagaccaggcatgag
PA5174 pZEN for NdeI ^a	gttatgcatatgacgcgaacgactattc
PA5174 pZEN rev XbaI ^a	gttatgtctagaggccttttccgttgtg
cvil P1 ^c	ggccagtgccaagctaggtgtgtcccaag
cvil P2 ^c	gtagagccgtctatgtacc
Gent for cvil ^c	catagacggctctaccgagctcgaattgac
Gent rev cvil ^c	gaaactgaccgttcggcttgaacgaattg
cvil P3 ^c	gaagcggctcagtttctctgc
cvil P4 ^c	acgaattcgagctcggatttacgctgctg

^a Cloned into vectors using PCR (overlap assembly if needed), DNA digestion, and T4 DNA ligase.

^b Cloned using the Gateway system (Invitrogen). ^c Cloned using the In-Fusion system (Clontech).

Table S2: *P. aeruginosa* PAO1 Cosmid Summary

Cosmid isolate	P. aeruginosa PAO1 Genome Coordinates		Insert length (bp)	Orientation ^c	Complements <i>Ec fabH</i> ^d
	Left end ^a	Right end ^b			
pTMYcos1	5825905	5859122	33217	R	Yes
pTMYcos2	5826015	5850856	24841	R	Yes
pTMYcos3	2576368	2611546	35178	R	No
pTMYcos4	5825903	5867229	41326	R	Yes
pTMYcos5	5826022	5861271	35249	R	Yes
pTMYcos6	5826020	5861972	35952	R	Yes
pTMYcos7	5826085	5860971	34886	R	Yes
pTMYcos8	5826052	5859591	33539	R	Yes
pTMYcos9	5826002	5866799	40797	R	Yes
pTMYcos10	5825889	5861887	35998	R	Yes
pTMYcos11	5825938	5863737	37799	R	Yes
pTMYcos12	3500948	3467718	33230	L	No
pTMYcos13	5863014	5826030	36984	L	Yes
pTMYcos14	5825990	5860787	34797	R	Yes
pTMYcos15	5825892	5859442	33550	R	Yes
pTMYcos16	2424390	2459310	34920	R	No
pTMYcos17	5826114	5868327	42213	R	Yes

^a Determined with pWEBdown primer. ^b Determined with pWEBup primer. ^c R- PA5174 is next to plasmid T7 promoter; L- PA5174 is next to M13 primer site. ^d Rescues growth of *fabH* depleted *E. coli* strain TMY19.

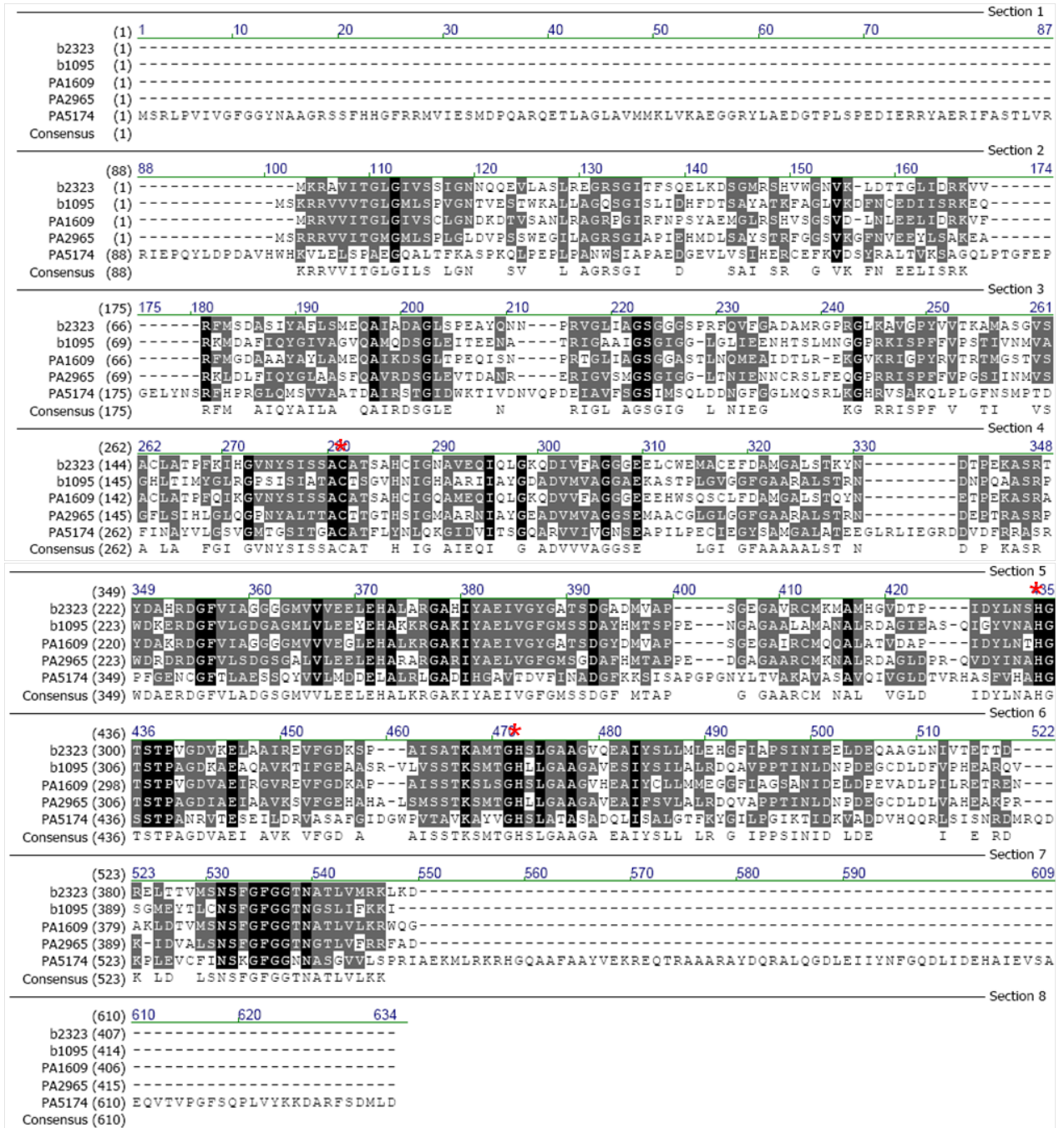
Table S3: Relative abundance of fatty acids in *P. aeruginosa* PAO1 KASIII domain ORF mutants ^a

Fatty acid	PAO1 wt NB52019 ^b	TMT15 ΔPA0998	TMT02 ΔPA0999	TMT01 ΔPA3333 ^b	TMT12 ΔPA3286 ^b	TMT16 Quad KO ^c
10:0 3OH	2.90±0.17	3.38	3.48	2.89±0.90	3.01±0.25	3.61
12:0	3.68±0.12	4.07	4.42	3.65±0.99	3.59±0.46	4.90
12:0 2OH	5.13±0.65	5.42	6.05	4.87±1.14	5.16±0.28	6.32
12:0 3OH	4.57±0.17	5.00	5.44	4.72±0.71	5.14±0.15	5.74
16:1 ω7c	9.68±0.07	10.08	9.58	11.75±0.09	8.75±1.34	11.05
16:0	33.73±0.54	33.25	33.17	31.92±0.50	31.4±1.05	31.24
17:0 cyclo ω7c	2.14±0.71	1.92	2.39	1.10±0.26	1.82±0.26	1.58
18:1 ω 7c	32.85±2.77	32.44	30.34	35.55±4.07	34.88±0.42	31.69
19:0 cyclo ω7c	2.95±0.80	2.44	3.02	1.28±0.04	3.85±2.21	1.85

^a Biomass scraped from LB agar plates. ^b From two independent experimental replicates.

^c ΔPA0998 ΔPA0999 ΔPA3333 ΔPA3286

Supplementary Figure 1



Supplementary Figure 1: Protein sequence alignment of KASI/II domain proteins in *P. aeruginosa* and *E. coli* MG1655. Identical amino acid residues are highlighted in black and conservative substitutions are in gray. Red asterisks denote active sites amino acid residues in *E. coli* FabB (Cys163-His-298-His-333) (86). b2323- *E. coli* MG1655 FabB; b1095- *E. coli* MG1655 FabF; PA1609- *P. aeruginosa* PAO1 FabB; PA2965- *P. aeruginosa* PAO1 FabF; PA5174- *P. aeruginosa* PAO1 FabY.