Supplementary Material for:

Fatty Acid Co-Substrates Provide β-Oxidation Precursors for Rhamnolipid Biosynthesis in *Pseudomonas aeruginosa*: Evidence from Isotope Tracing and Gene Expression

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Three isotopologues of Acetyl-CoA (d_0, d_1 and d_3) and three isotopologues of malonyl-ACP (d_0, d_1 and d_2) can theoretically be found in the *de novo* fatty acid synthesis substrate pool in the 1% glucose + 0.25% octadecanoic acid-d_{35} treatment, if it is assumed that d_{35}-octadecanoic acid is completely degraded through β-oxidation and generates 9 acetyl-CoAs [8 acetyl-CoA (d_1) and 1 acetyl-CoA (d_3)]:

- Acetyl-CoA (d_0) is generated from the metabolism of glucose.
- Acetyl-CoAs (d_1 and d_3) are generated from the catabolism of d_{35}-octadecanoic acid through β-oxidation.
- Malonyl-ACP (d_0) is formed by adding a molecule of bicarbonate with a molecule of either acetyl-CoA (d_0) or acetyl-CoA (d_1) following by a CoA: ACP transacylation reaction.
- Malonyl-ACP (d_1) is formed by adding a molecule of bicarbonate with a molecule of acetyl-CoA (d_1) following by a CoA: ACP transacylation reaction.
- Malonyl-ACP (d_2) is formed by adding a molecule of bicarbonate with a molecule of acetyl-CoA (d_3) following by a CoA: ACP transacylation reaction.

Table S1 Isotope tracing results for the synthesis of (R)-β-hydroxydecanoyl-ACP through de novo fatty acid synthesis pathway. "×" means that there is a chance to synthesize (R)-β-hydroxydecanoyl-ACP (d_n).

Initial Cycle (Cycle 1)		Elongation Cycle			(R)-β-hydroxydecanoyl-ACP (d_n)								
		Cycle 2	Cycle 3	Cycle 4									
Acetyl-CoA (d_n)	Malonyl-ACP (d_n)	Malonyl-ACP (d_n)	Malonyl-ACP (d_n)	Malonyl-ACP (d_n)	d_0	d_1	d_2	d_3	d_4	d_5	d_6	d_7	d_8
d_0	d_0	d_0	d_0	d_0	×								
				d_1		×							
				d_2			×						
			d_1	d_0	×	×							
				d_1		×	×						
				d_2			×	×					
			d_2	d_0		×							
				d_1			×						
				d_2				×					
		d_1	d_0	d_0	×	×							
				d_1		×	×						
				d_2			×	×					
			d_1	d_0	×	×	×						
				d_1		×	×	×					
				d_2			×	×	×				
			d_2	d_0		×	×						
				d_1			×	×					
				d_2				×	×				

		d_2	d_0	d_0		×							
				d_1			×						
				d_2				×					
			d_1	d_0		×	×						
				d_1			×	×					
				d_2				×	×				
			d_2	d_0			×						
				d_1				×					
				d_2					×				
	d_1	d_0	d_0	d_0	×	×							
				d_1		×	×						
				d_2			×	×					
			d_1	d_0	×	×	×						
				d_1		×	×	×					
				d_2			×	×	×				
			d_2	d_0		×	×						
				d_1			×	×					
				d_2				×	×				
		d_1	d_0	d_0	×	×	×						
				d_1		×	×	×					
				d_2			×	×	×				
			d_1	d_0	×	×	×	×					
				d_1		×	×	×	×				
				d_2			×	×	×	×			
			d_2	d_0		×	×	×					
				d_1			×	×	×				
				d_2				×	×	×			

		d_2	d_0	d_0		×	×						
				d_1			×	×					
				d_2				×	×				
			d_1	d_0		×	×	×					
				d_1			×	×	×				
				d_2				×	×	×			
			d_2	d_0			×	×					
				d_1				×	×				
				d_2					×	×			
	d_2	d_0	d_0	d_0		×							
				d_1			×						
				d_2				×					
			d_1	d_0		×	×						
				d_1			×	×					
				d_2				×	×				
			d_2	d_0			×						
				d_1				×					
				d_2					×				
		d_1	d_0	d_0		×	×						
				d_1			×	×					
				d_2				×	×				
			d_1	d_0		×	×	×					
				d_1			×	×	×				
				d_2				×	×	×			
			d_2	d_0			×	×					
				d_1				×	×				
				d_2					×	×			

		d_2	d_0	d_0			×						
				d_1				×					
				d_2					×				
			d_1	d_0			×	×					
				d_1				×	×				
				d_2					×	×			
			d_2	d_0				×					
				d_1					×				
				d_2						×			
d_1	d_0	d_0	d_0	d_0		×							
				d_1			×						
				d_2				×					
			d_1	d_0		×	×						
				d_1			×	×					
				d_2				×	×				
			d_2	d_0			×						
				d_1				×					
				d_2					×				
		d_1	d_0	d_0		×	×						
				d_1			×	×					
				d_2				×	×				
			d_1	d_0		×	×	×					
				d_1			×	×	×				
				d_2				×	×	×			
			d_2	d_0			×	×					
				d_1				×	×				
				d_2					×	×			

		d_2	d_0	d_0			×						
				d_1				×					
				d_2					×				
			d_1	d_0			×	×					
				d_1				×	×				
				d_2					×	×			
			d_2	d_0				×					
				d_1					×				
				d_2						×			
	d_1	d_0	d_0	d_0		×	×						
				d_1			×	×					
				d_2				×	×				
			d_1	d_0		×	×	×					
				d_1			×	×	×				
				d_2				×	×	×			
			d_2	d_0			×	×					
				d_1				×	×				
				d_2					×	×			
		d_1	d_0	d_0		×	×	×					
				d_1			×	×	×				
				d_2				×	×	×			
			d_1	d_0		×	×	×	×				
				d_1			×	×	×	×			
				d_2				×	×	×	×		
			d_2	d_0			×	×	×				
				d_1				×	×	×			
				d_2					×	×	×		

		d_2	d_0	d_0			×	×					
				d_1				×	×				
				d_2					×	×			
			d_1	d_0			×	×	×				
				d_1				×	×	×			
				d_2					×	×	×		
			d_2	d_0				×	×				
				d_1					×	×			
				d_2						×	×		
	d_2	d_0	d_0	d_0			×						
				d_1				×					
				d_2					×				
			d_1	d_0			×	×					
				d_1				×	×				
				d_2					×	×			
			d_2	d_0				×					
				d_1					×				
				d_2						×			
		d_1	d_0	d_0			×	×					
				d_1				×	×				
				d_2					×	×			
			d_1	d_0			×	×	×				
				d_1				×	×	×			
				d_2					×	×	×		
			d_2	d_0				×	×				
				d_1					×	×			
				d_2						×	×		

		d_2	d_0	d_0				×					
				d_1					×				
				d_2						×			
			d_1	d_0				×	×				
				d_1					×	×			
				d_2						×	×		
			d_2	d_0					×				
				d_1						×			
				d_2							×		
d_3	d_0	d_0	d_0	d_0				×					
				d_1					×				
				d_2						×			
			d_1	d_0				×	×				
				d_1					×	×			
				d_2						×	×		
			d_2	d_0					×				
				d_1						×			
				d_2							×		
		d_1	d_0	d_0				×	×				
				d_1					×	×			
				d_2						×	×		
			d_1	d_0				×	×	×			
				d_1					×	×	×		
				d_2						×	×	×	
			d_2	d_0					×	×			
				d_1						×	×		
				d_2							×	×	

		d_2	d_0	d_0					×				
				d_1						×			
				d_2							×		
			d_1	d_0					×	×			
				d_1						×	×		
				d_2							×	×	
			d_2	d_0						×			
				d_1							×		
				d_2								×	
	d_1	d_0	d_0	d_0				×	×				
				d_1					×	×			
				d_2						×	×		
			d_1	d_0				×	×	×			
				d_1					×	×	×		
				d_2						×	×	×	
			d_2	d_0					×	×			
				d_1						×	×		
				d_2							×	×	
		d_1	d_0	d_0				×	×	×			
				d_1					×	×	×		
				d_2						×	×	×	
			d_1	d_0				×	×	×	×		
				d_1					×	×	×	×	
				d_2						×	×	×	×
			d_2	d_0					×	×	×		
				d_1						×	×	×	
				d_2							×	×	×

		d_2	d_0	d_0					×	×			
				d_1						×	×		
				d_2							×	×	
			d_1	d_0					×	×	×		
				d_1						×	×	×	
				d_2							×	×	×
			d_2	d_0						×	×		
				d_1							×	×	
				d_2								×	×
	d_2	d_0	d_0	d_0					×				
				d_1						×			
				d_2							×		
			d_1	d_0					×	×			
				d_1						×	×		
				d_2							×	×	
			d_2	d_0						×			
				d_1							×		
				d_2								×	
		d_1	d_0	d_0					×	×			
				d_1						×	×		
				d_2							×	×	
			d_1	d_0					×	×	×		
				d_1						×	×	×	
				d_2							×	×	×
			d_2	d_0						×	×		
				d_1							×	×	
				d_2								×	×

		d_2	d_0	d_0						×			
				d_1							×		
				d_2								×	
			d_1	d_0						×	×		
				d_1							×	×	
				d_2								×	×
			d_2	d_0							×		
				d_1								×	
				d_2									×

Table S2 Possibility of producing (R)-β-hydroxydecanoyl-ACP (d_n) through *de novo* fatty acid synthesis, based on the assumption that all possible reactions in the *de novo* cycles have the same chance to happen.

(R)-β-hydroxydecanoyl-ACP (d_n)	d_0	d_1	d_2	d_3	d_4	d_5	d_6	d_7	d_8
Number of Ways	8	35	73	100	100	81	54	27	8
Possibility (%)	1.6	7.2	15.0	20.6	20.6	16.7	11.1	5.6	1.6

Table S3 Intermediates (chain length and deuteration) of octadecanoic acid-d_{35} β-oxidation and acetyl-CoAs (d_n) produced

β-oxidation cycle	Acyl-CoA (d_n)	Trans-2,3-enoyl-CoA (d_n)	S-β-hydroxyacyl-CoA (d_n)	β-ketoacyl-CoA (d_n)	Acetyl-CoA (d_n)
Cycle 1	C_{18} (d_{35})	C_{18} (d_{33})	C_{18} (d_{33})	C_{18} (d_{32})	C_2 (d_1)
Cycle 2	C_{16} (d_{31})	C_{16} (d_{29})	C_{16} (d_{29})	C_{16} (d_{28})	C_2 (d_1)
Cycle 3	C_{14} (d_{27})	C_{14} (d_{25})	C_{14} (d_{25})	C_{14} (d_{24})	C_2 (d_1)
Cycle 4	C_{12} (d_{23})	C_{12} (d_{21})	C_{12} (d_{21})	C_{12} (d_{20})	C_2 (d_1)
Cycle 5	C_{10} (d_{19})	C_{10} (d_{17})	C_{10} (d_{17})	C_{10} (d_{16})	C_2 (d_1)
Cycle 6	C_8 (d_{15})	C_8 (d_{13})	C_8 (d_{13})	C_8 (d_{12})	C_2 (d_1)
Cycle 7	C_6 (d_{11})	C_6 (d_9)	C_6 (d_9)	C_6 (d_8)	C_2 (d_1)
Cycle 8	C_4 (d_7)	C_4 (d_5)	C_4 (d_5)	C_4(d_4)	C_2 (d_1) and C_2 (d_3)