

Supplementary Table S1. Primers used in this study

k/o 6246 F-SmaI	5'-ACCCGGGCTAGACGGGCAGCACG-3'
k/o 6246 R-BamHI	5'-ATGGATCCTCAGTCCAGACCCAGGC-3'
k/o 6247 F-KpnI	5'-AAGGTACCGAACCCCAAGGCGATAGT-3'
k/o 6247 R-BamHI	5'-ATGGATCCTCAGTCCAGACCCAGGC-3'
k/o 6248 F-SacI	5'-ATGAGCTCTCTCCATCATGATCGGCACT-3'
k/o 6248 R-PstI	5'-AACTGCAGATCGACGCTCTGCTCGCA-3'
k/o 6249 F-SacI	5'-TAGAGCTCGGTGGCCTGGATAGACTGCCT-3'
k/o 6249 R-BamHI	5'-TAGGATCCAGGAACAGGGACGCGCCGATCA-3'
k/o 6250 F-BamHI	5'-ACGGATCCATGAAAAAGATCCTGATC-3'
k/o 6250 R-HindIII	5'-TTAAGCTTTCAGTCCACCGCTAGGAG-3'
k/o 6251 F-MfeI	5'-AACAAATTGGAACGCCGCACCGAACAT-3'
k/o 6251 R-XbaI	5'-TTTCTAGACTACGCCGCCGAGCCCA-3'
PsPA7_6248 F-SmaI	5'-TACCTGCCCCGGGATGGAAGTCTTCGGCT-3'
PsPA7_6248 R-PstI	5'-CGATCTGCAGTCAGTCCAGACCCAGGC-3'
PA1416 F-StuI	5'-ATCAGGCCTGACCCGGAGCCC-3'
PA1416 R-PstI	5'-AACTGCAGTCAGGGTATGACCTTG-3'
MSMEG_6382 F-NcoI	5'-TACCATGGATGGGCGCGGTAC-3'
MSMEG_6382 R-HindIII	5'-TTAAGCTTTCAGAGCAGTTGCAGGC-3'
Rv3790 F-NcoI	5'-TACCATGGATGTTGAGCGTGGGA-3'
Rv3790 R-HindIII	5'-ACAAGCTTCTACAGCAGCTCCAAG-3'
6245-6251 F-SmaI	5'-ACCCGGGCTAGACGGGCAGCACG-3'
6245-6251 R-XbaI	5'-TTTCTAGACTACGCCGCCGAGCCCA-3'
6247-6251 F-SmaI	5'-ACCCGGGAAGAGTTGGTGTGATGAAGCTGGC-3'
6247-6251 R-XbaI	5'-TTTCTAGACTACGCCGCCGAGCCCA-3'
6245-6249 F-SmaI	5'-ACCCGGGCTAGACGGGCAGCACG-3'

6245-6249 R-XbaI	5'-AATCTAGATCAGAGGCTGAGCTTCTTGAAAAG-3'
6246-6249 F-SmaI	5'-AACCCGGGTCAGTGCGGCCAGAGCG-3'
6246-6249 R-XbaI	5'-AATCTAGATCAGAGGCTGAGCTTCTTGAAAAG-3'

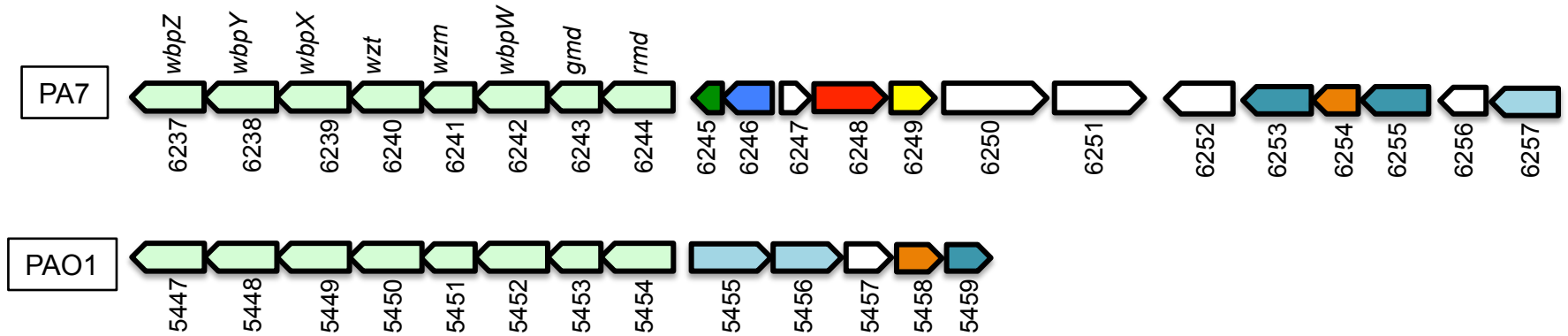
Supplementary Fig. S1. Genomic context of the genes involved in D-Araf biosynthesis in *P. aeruginosa*. *A.* In PA7, the DPA biosynthetic genes are adjacent to the A-band LPS biosynthetic genes (light green). Specific gene numbers for PA7 (PsPA7_xxxx) and PAO1 (PAxxxx) are shown below the gene map, while the conventional names are shown above (20). In PAO1, the same position is occupied by another putative polysaccharide biosynthetic cluster (PA5455-5459). Homologues of a subset of those ORFs can be identified downstream of the D-Araf biosynthetic cluster by homology searches of the PA7 genome, although they are oriented on the opposite strand. Genes encoding similar products are similarly coloured in blue and orange; genes in white have no homologues. *B.* Alignment of Rmd proteins from PAO1 and PA7. Rmd is highly conserved among *P. aeruginosa* strains with the exception of PA7. The N-terminus of PA7 Rmd is less conserved than the C-terminus, suggesting that the D-Araf biosynthetic genes may have been acquired through horizontal gene transfer and homologous recombination within the *rmd* locus.

Supplementary Fig. S2. Alignment of polyprenyl-P-D-ribose-5-P synthases (PPPRS). PPPRS proteins from *Azorhizobium caulinodans* (NoeC), *P. syringae* pv *syringae* (P syr), *M. tuberculosis* H37Rv (Mtb) and *P. aeruginosa* PA7 were aligned in Geneious (Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A (2010) Geneious v5.0, <http://www.geneious.com>) using the MUSCLE algorithm. Identical residues are shown in reverse text, while highly conserved residues are highlighted in grey. The putative active site motif NDxxD found in other polyprenyl transferases such as UbiA (33,34) is boxed in red. Residues marked with red asterisks were previously shown by site-directed mutagenesis to be important for activity of the *M. tuberculosis* enzyme (33), while the residue marked with a blue asterisk was not required, consistent with its lack of conservation in PA7.

Supplementary Fig. S3. Alignment of DprE1 proteins. DprE1 proteins from *P. aeruginosa* PA7, *M. tuberculosis* and *M. smegmatis* were aligned in Geneious (Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A (2010) Geneious v5.0, <http://www.geneious.com>) using the MUSCLE algorithm. The key Cys residue that is required for sensitivity of the enzyme to benzothiazinones and dinitrobenzamides (13-15,28) is boxed in red and highlighted with an asterisk. The PA7 enzyme has an Ala at the corresponding position, suggesting it would be resistant to inactivation.

Harvey et al., Supplementary Figure S1

A.



Identities = 251/304 (82%), Positives = 272/304 (89%), Gaps = 1/304 (0%)

B.

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PAO1: 1  MTQRLFVTGLSGFVGKHLQAYLAAAHTPWALLPVPHRYDLLEPDSLGLWPELPDAVIHL 60
      M + L VTGL+GFVG+HL+ + + PW+LLP P +DL P+SL E+PDAVIHL
PA7: 1  MKKSLLVVTGLNGFVGRHLRQRIESHDLPWSLLPHPA-FDLAVPESLESWRCEMPDAVIHL 59

PAO1: 61 AGQTYVPEAFRDPARTLQINLLGTLNLLQALKARGFSGTFLYISSGDVYGQVAEALPIH 120
      AGQT+VP++F DP RT ++N LGTL+LLQALK GF+GTFLYISSGDVYGQV EAALPIH
PA7: 60 AGQTFVPDSFLDPRRTFEVNFLGTLSSLQALKRNGFAGTFLYISSGDVYGQVPEALPIH 119

PAO1: 121 EELIPHPRNPYAVSKLAAESLCLQWGITEGWRVLRPFNHIGPGQKDSFVIASAARQIA 180
      EE +P PRNPYAVSKLAAESLCLQWGI+EGWRVLRPFNHIGPGQKDSFVIASAARQIA
PA7: 120 EEFLPRPRNPYAVSKLAAESLCLQWGISEGWRVLRPFNHIGPGQKDSFVIASAARQIA 179

PAO1: 181 RMKQGLQANRLEVGDIDVSRDFLDVQDVL SAYLRLLSHGEAGAVYNVCSGQE QKIRELIE 240
      RMKQGLQA+RLEVGDIDVSRDFLDVQDVL SAYLRLLSHGE GAVYNVCSGQE QKIR+LIE
PA7: 180 RMKQGLQ AHRLEVGDIDVSRDFLDVQDVL SAYLRLLSHGEPGAVYNVCSGQE QKIRD LIE 239

PAO1: 241 LLADIAQVELEIVQD PARMRRAEQRRV R GSHARLHDTTGWKPEITIKQSLRAILSDWESR 300
      LLADIAQVELEIVQD PARMRRAEQRRV GSHARL D TGWKPEITIKQSLRAILSDWESR
PA7: 240 LLADIAQVELEIVQD PARMRRAEQRRVCGSHARLRDATGWKPEITIKQSLRAILSDWESR 299

PAO1: 301 VREE 304
      VREE
PA7: 300 VREE 303
    
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Harvey et al., Supplementary Figure S2

- 1. NoeC from Azorhizobium
- 2. PPRS from Psyr
- 3. DPPRS from Mtb
- 4. PPRS from PA7

1 10 20 30 40 50 60 70
M P G T O G R A L E V D T P L V V D L D G T L L R S D L L F E T A V A F I R G R P L O V F R L F T W L L O G K A P L K O G L A L G T D I D V A L

- 1. NoeC from Azorhizobium
- 2. PPRS from Psyr
- 3. DPPRS from Mtb
- 4. PPRS from PA7

80 90 100 110 120 130 140
L P Y D A A V I A Y I Q T S R Q H G R R V V L A T A S H E T L A N Q I A A H L Q M F D Q V W A S D G K T N L S A H R K R D L L V S H Y G E G G F F
 M W N K E W A A K I P -----
 M S E D V V T Q P P -----
M L R S P P T T C R S G P A S S P -----
 ----- T

- 1. NoeC from Azorhizobium
- 2. PPRS from Psyr
- 3. DPPRS from Mtb
- 4. PPRS from PA7

150 160 170 180 190 200 210
 M T Y -----
 D Y I G N S R D D L C I W K V S R K A I V A S P L A G V E R A A R A Q G N V E Q V I K S T S S R R S A W Y K A L R L H Q W L K N T L I F - V P L
 N L V -----
 L P V P A T R G Q V G M H T T V E R S S L A Q L K G L -----
 L A L M R E R Q W V K N A F V V - A P L

- 1. NoeC from Azorhizobium
- 2. PPRS from Psyr
- 3. DPPRS from Mtb
- 4. PPRS from PA7

220 230 240 250 260 270 280
L I C G R A E - - D L - - L Q A P L W L A F M T F C S V A S G I Y V I N D L M D R A H D R R H P S K R H - R P F A S R K L S G L T G V W M C L V
 L A A H Q V Q S T Q L - - L L D G T - L A F L C F G L C A S S V Y L I N D L L D L A D D R H H R S K R E - R P F A - - - S G Q L S I E S G L L
 A A L G G V R Y D Y V E V L S K V S M A F V V F S L A S A V Y L V N D V R D V E A D R E H P T K R F - R P F A - - - A G V V P E W L A Y T
 L F S G E F L Q V D A - - V L H A L - A A V L F C L A S S A T Y V I N D L H D L E R D R L H P K K A L T R P L A - - - S G L V S P L Q A K Y

- 1. NoeC from Azorhizobium
- 2. PPRS from Psyr
- 3. DPPRS from Mtb
- 4. PPRS from PA7

290 300 310 320 330 340 350 360
L I A I G G V C A I N C - - - - G E R L F A I T A S Y V A L S V I Y V G K V R G E Y V L D L F V L S A I Y T T R I I A G A T A A N I P V P A S
V I F I L L A A A F A G A A I M L P W Q F A A V L A A Y Y L L T L V Y S L Y L K R H M A V D V I V L A M I Y T T R I I A G A A A F Q L P L T F W
V A V V L G V T S L A G - A W M L T P N L A I M V M V V Y L A M O L A Y C F G L K H Q A V V E I C V S S A Y L I R A I A G V A T K I P L S K W
L L A I L Y L A L L G G - - L L T S P P V I M V I G L Y L L N L A Y T F F L K H Q P V L D I F T I A L G F V L R V Y A G A V A L A V P V S S W

- 1. NoeC from Azorhizobium
- 2. PPRS from Psyr
- 3. DPPRS from Mtb
- 4. PPRS from PA7

370 380 390 400 410 420 430
F L A F S A M A F V S L A S I K R L N E L T Q L R R D G A P - D L Y G R G Y E L S D H S I V A L I C V S A G Y A A V V F L E L F V Q M S S - - -
I L A F S M F L F L S L A L V K R Y A E I R D A R L C D V T V K T R G R G Y Y P G D L D M I A S L G A S S G N L A V M V L A L Y I H E G A T V A
F L L I M A F G S L F M V A G K R Y A E I H L A E R T G A A I R K S L E S Y T S T Y L R F V W T L S A T - - - A V V I C Y G L W A F E R D - - -
M F V T T L C L A L Y L A A V K R Q E L A - - - R N G S Q S R E V L Q H Y S L A L V D R Y A E M S A T - - - G A L L F Y S L F V I T S R - - -

- 1. NoeC from Azorhizobium
- 2. PPRS from Psyr
- 3. DPPRS from Mtb
- 4. PPRS from PA7

440 450 460 470 480 490 500
V A Q G P A P I F V S N A M C V V V A Y W I S R A V - V Q A H R G D M R S D T L C Y A V T D G S S L V C I L G L A L G L V F L M Y C - R S Q S I
L Y Q H P H V I W L A - - - C P L L E W I T T R I W - M L T H R G O M N E D E V V F A I R D - - - - - R I S Q G I G F L L L V F - - - - W I
- - - G Y S G S W F A - - - V S M T P E T H A I L R Y A V D V D G G L A G E P E D I A L R D - - - - - R V L Q L L A L A W I A T V G A A V A F
- - - - - E E L V V T - - - I P L V L E G L F R Y W Y V V E A K E G - G E S P T D A L L S D - - - - - W Q L L A T V V L W G L A C A Y A L W P

- 1. NoeC from Azorhizobium
- 2. PPRS from Psyr
- 3. DPPRS from Mtb
- 4. PPRS from PA7

506
 G
A A
 G
 H

Harvey et al., Supplementary Figure S3

	1	10	20	30	40	50	60	70																																																																			
1. DprE1 PA7			M	F	V	F	G	W	G	R	Y	P	R	I	N	A	T	L	V	S	P	R	S	V	D	E	L	R	R	R	I	Q	L	S	S	-	-	-	-	-	G	I	T	L	R	G	H	G	K	S	Y	G	D	S	A	L	-	-																	
2. DprE1 smegmatis	M	G	A	V	P	S	L	T	M	S	T	T	E	F	P	T	T	T	K	R	M	G	W	G	R	T	A	P	T	V	A	S	V	L	S	T	S	D	P	E	V	I	V	R	A	V	T	R	A	A	E	E	-	G	G	R	G	V	I	A	R	G	L	G	R	S	Y	G	D	N	A	Q	N	G	
3. DprE1 tuberculosis	M	L	S	V	G	A	T	-	-	-	T	T	A	T	R	L	T	G	W	G	R	T	A	P	S	V	A	N	V	I	R	T	P	D	A	E	M	I	V	K	A	V	A	R	V	A	E	S	G	G	R	G	A	I	A	R	G	L	G	R	S	Y	G	D	N	A	Q	N	G						
1. DprE1 PA7		80		90		100		110		120		130		140		150																																																											
2. DprE1 smegmatis	G	E	S	T	A	D	S	R	N	L	D	H	L	O	S	F	G	E	P	T	G	V	L	R	C	F	A	G	T	T	L	A	D	L	A	A	T	F	L	P	R	G	W	F	L	P	V	T	P	G	T	A	H	I	S	V	G	G	A	I	A	S	D	V	H	G	K	N	H	H	L	H	C	F	
3. DprE1 tuberculosis	G	G	L	V	I	D	M	P	A	L	N	R	I	H	S	I	D	S	G	T	R	L	V	D	V	D	A	G	V	S	L	D	Q	L	M	K	A	A	L	P	H	G	L	W	V	P	V	L	P	G	T	R	Q	V	T	V	G	G	A	I	C	D	I	H	G	K	N	H	H	S	A	G	S	F	
1. DprE1 PA7		160		170		180		190		200		210		220																																																													
2. DprE1 smegmatis	S	E	F	V	D	S	F	R	L	L	A	D	G	D	L	H	-	C	S	R	N	E	H	P	E	L	F	H	A	T	C	G	G	M	G	L	T	G	A	L	V	D	V	T	L	R	L	R	R	V	P	S	A	W	I	D	Q	V	T	C	K	A	N	N	L	E	E	A	F	E	L	F	E		
3. DprE1 tuberculosis	G	N	H	V	R	S	M	E	L	L	T	A	N	G	E	V	R	H	L	T	P	A	G	P	D	S	D	L	E	W	A	T	V	G	G	N	G	L	T	G	I	I	L	R	A	T	I	E	M	T	P	T	E	T	A	Y	F	I	A	D	G	D	V	T	G	S	L	D	E	T	I	A	F	H	S
1. DprE1 PA7		230		240		250		260		270		280		290		300																																																											
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3. DprE1 tuberculosis	D	G	S	E	A	N	Y	T	Y	S	S	A	W	F	D	A	I	S	K	P	P	K	L	G	R	A	A	T	S	R	G	S	L	A	K	L	D	Q	L	P	S	K	L	Q	K	D	P	L	K	F	D	A	P	Q	L	L	T	L	P	D	I	F	P	N	G	L	A	N	K	F	T	F	M	P	I
1. DprE1 PA7		310		320		330		340		350		360		370																																																													
2. DprE1 smegmatis	N	S	L	Y	F	O	R	I	R	Q	P	R	V	E	O	R	V	D	Y	R	S	F	F	Y	P	L	D	G	I	G	D	W	N	M	Y	G	R	N	C	F	L	O	Y	O	F	V	I	P	A	S	A	G	L	P	G	M	R	R	I	L	E	R	I	A	A	S	G	R	G	S	F	L	A	V	
3. DprE1 tuberculosis	G	E	L	W	Y	R	K	S	G	T	Y	R	N	K	V	O	-	N	L	T	O	F	Y	H	P	L	D	M	F	G	E	W	N	R	A	Y	G	S	A	G	F	L	O	Y	O	F	V	I	P	T	E	A	-	V	E	E	F	K	S	I	I	V	D	I	Q	R	S	C	H	Y	S	F	L	N	V
1. DprE1 PA7		380		390		400		410		420		430		440		450																																																											
2. DprE1 smegmatis	L	K	A	F	G	E	G	N	E	N	P	L	S	F	P	Q	K	G	Y	T	A	L	D	F	K	M	D	A	S	L	L	P	L	L	D	E	L	D	R	R	V	L	E	F	G	G	R	L	Y	L	A	K	D	A	R	M	S	E	A	T	F	K	Q	S	Y	P	R	W	E	N	F	O	E	T	
3. DprE1 tuberculosis	F	K	L	F	G	P	G	N	O	A	P	L	S	F	P	I	P	G	W	N	V	C	V	D	F	P	T	K	A	G	T	H	E	F	V	T	E	L	D	R	R	V	L	E	F	G	G	R	L	Y	T	A	K	D	S	R	T	A	E	T	F	H	A	M	Y	P	R	I	D	E	W	I	R		
1. DprE1 PA7		460		471																																																																							
2. DprE1 smegmatis	R	R	Y	G	A	L	G	K	F	T	S	L	O	A	R	R	L	G	L	D																																																							
3. DprE1 tuberculosis	R	R	K	V	D	P	L	R	V	F	A	S	D	M	A	R	R	L	E	L																																																							