

- 1 **Table S1A. Details of SNP mutations found in *P. aeruginosa* DP evolved populations.** Mutations observed in control populations were
- 2 removed to avoid the effect of long-term growth on LB. Gray shading indicates fixed mutations and gray shading with grid indicates
- 3 mutations with less than 80% frequency (Breseq consensus mode).

Contig	Annotation	Gene position (Contig)	Mutation in contig	Amino acid change	BAC(+)_1	BAC(+)_2	BAC(+)_3	BAC(-)_1	BAC(-)_2	BAC(-)_3
Cont130	tolQ-type transporter	10,800	C→A	Glu → Stop						
Cont130	ribonuclease E	5,273	A→G	Silent						
Cont147	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexD	66,759	T→C	Asn → Ser						
Cont147	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexD	65,685	A→C	Val → Gly						
Cont174	Type III secretion outer membrane protein PopN precursor	100,586	C→T	Silent						
Cont246	hypothetical protein H123_32466, partial	25	A→G	Silent						
Cont41	hemagglutination protein, partial	52,142	G→A	Silent						
Cont53	PmrB: two-component	8,288	A→G	Phe →						

	regulator system signal sensor kinase PmrB			Leu						
Cont53	PmrB: two-component regulator system signal sensor kinase PmrB	8,636	T→C	Met → Val						
Cont94	aluminum activated malate transporter family protein	33,856	G→A	Thr → Ile						
Cont188	Full=UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase; AltName: Full=Protein EnvA; AltName: Full=UDP-3-O-acyl-GlcNAc deacetylase	80,476	A→T	Leu → Gln						
Cont188	Full=RNA polymerase sigma-54 factor	134,734	T→A	Trp → Arg						
Cont240	hypothetical protein of bacteriophage Pfl	739	C→T	Silent						
Cont245	glycoprotein, partial	1,247	C→T	Ala → Thr						
Cont224	glycoprotein, partial	1,031	G→A	Silent						
Cont55	hypothetical protein PA0943	63,040	G→C	Tyr → Stop						
Cont55	lipopolysaccharide biosynthetic protein LpxO2	58,466	G→C	Arg → Gly						

Cont99	Full=DNA-directed RNA polymerase subunit alpha; Short=RNAP subunit alpha; AltName: Full=RNA polymerase subunit alpha; AltName: Full=Transcriptase subunit alpha	13,252	C→G	Leu → Val						
Cont195	transposase insM for insertion sequence element IS600	625	G→A	Silent						
Cont195	transposase insM for insertion sequence element IS600	1,180	A→G	Silent						
Cont195	transposase insM for insertion sequence element IS600	903	A→G	Lys → Arg						
Cont210	alpha-1,6-rhamnosyltransferase	12,406	A→C	His → Pro						
Cont44	hypothetical protein PA3904	10,230	G→A	Silent						
Cont85	RecName: Full=Chaperone SurA; AltName: Full=Peptidyl-prolyl cis-trans isomerase SurA; Short=PPIase SurA; AltName: Full=Rotamase	67,351	T→G	Thr → Pro						

	SurA; Flags: Precursor									
	RecName: Full=Chaperone SurA; AltName: Full=Peptidyl- prolyl cis-trans isomerase SurA; Short=PPIase SurA; AltName: Full=Rotamase SurA; Flags: Precursor	21,278	C→T	Silent						
Cont142	lipopolysaccharide biosynthetic protein LpxO1	12,214	A→G	His → Arg						
Cont165	peptidase S41	67,419	G→A	Gly → Ser						
Cont56	type IV secretion protein Rhs	1,398	T→C	Silent						
Cont84	RecName: Full=Denitrification regulatory protein NirQ	3,964	G→A	Silent						

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7 **Table S1B. Details of SNP mutations in *P. aeruginosa* DPB evolved populations.** Mutations observed in control populations were
8 removed to avoid the effect of long-term growth on LB. Gray shading indicates fixed mutations and gray shading with grid indicates
9 mutations with less than 80% frequency (Breseq consensus mode).

Contig	Annotation	Gene position (Contig)	Mutation in contig	Amino acid change	BAC(+)_1	BAC(+)_2	BAC(+)_3	BAC(-)_1	BAC(-)_2	BAC(-)_3
Cont110	two-component response regulator CbrB	38,368	T→C	Ile→Thr						
Cont110	PmrB: two-component regulator system signal sensor kinase PmrB	94,923	G→A	Val→Ile						
Cont110	PmrB: two-component regulator system signal sensor kinase PmrB	95,407	T→C	Leu→Pro						
Cont110	PmrB: two-component regulator system signal sensor kinase PmrB	95,266	G→C	Gly→Ala						
Cont64	glycoprotein, partial	21,505	C→G	Silent						
Cont67	cbb3-type cytochrome c oxidase subunit 1	149,131	C→G	Silent						
Cont67	cbb3-type cytochrome c oxidase subunit 1	149,266	C→G	Silent						
Cont77	serine protease MucD	61,376	A→G	Val→Ala						
Cont98	hemagglutination protein, partial	52,144	G→A	Silent						

Cont98	hemagglutination protein, partial	52,205	C→A	Pro→Lys						
Cont98	hemagglutination protein, partial	52,206	C→A							
Cont104	RecName: Full=Multidrug efflux pump subunit AcrB; AltName: Full=AcrAB-TolC multidrug efflux pump subunit AcrB; AltName: Full=Acridine resistance protein B	367	C→G	Silent						
Cont52	pyoverdine sidechain peptide synthetase, partial	87	C→G	Silent						
Cont108	mammalian cell entry protein	19,461	C→A	Asp→Tyr						
Cont112	Carbamoyltransferase in large core OS assembly cluster	84,231	T→G	Asp→Ala						
Cont43	TetR family transcriptional regulator	217,693	G→A	Gln→Stop						
Cont85	chloramphenicol acetyltransferase	23,702	A→C	Thr→Pro						
Cont75	MULTISPECIES: dehydrogenase [Pseudomonas], hypothetical protein PA0943 [Pseudomonas	28,392	G→A	Trp→Stop						

	aeruginosa PAO1]									
Cont92	Full=UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase; AltName: Full=Protein EnvA; AltName: Full=UDP-3-O-acyl-GlcNAc deacetylase	94,358	A→G	Ile→Thr						
Cont27	outer membrane protein assembly complex, YaeT protein	75,983	T→C	Gln→Arg						
Cont27	phosphoesterase	481,483	A→C	Thr→Pro						
Cont42	RecName: Full=Protease HtpX; AltName: Full=Heat shock protein HtpX	290,119	A→G	Ser→Pro						

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14 **Table S1C. Details of DIP (deletion, insertion and other polymorphisms) mutations detected in *P. aeruginosa* DP evolved**

15 **populations.** Mutations observed in control populations were removed avoid the effect of long-term growth on LB. Gray shading indicates

16 fixed mutations and gray shading with grid indicates mutations with less than 80% frequency (Breseq consensus mode).

Contig	Annotation	Gene position (Contig)	Mutation in contig	BAC(+)_1	BAC(+)_2	BAC(+)_3	BAC(-)_1	BAC(-)_2	BAC(-)_3
Cont130	tolQ-type transporter	10,940	+TGGCGG						
Cont174	RecName: Full=Uroporphyrinogen-III C-methyltransferase; Short=Urogen III methylase; AltName: Full=SUMT; AltName: Full=Uroporphyrinogen III methylase; Short=UROM	25,207	Δ36 bp						
Cont125	peptidase S41	1,654	G→.						
Cont125	peptidase S41	1,537	+A						
Cont181	enoyl-CoA hydratase	1,397	Δ40 bp (large del)						
Cont38	TetR family transcriptional regulator	18,939	191 bp x 2						
Cont16	sugar-binding protein	19,119	A→.						
Cont162	hydrogenase expression protein HypA, multidrug efflux protein, RND efflux system, inner membrane transporter	21,318	Δ387 bp						

	CmeB								
Cont162	hemolysin D, periplasmic multidrug efflux lipoprotein, RND multidrug efflux membrane fusion protein precursor, RND efflux system, membrane fusion protein CmeA	19,001	C→.						
Cont184	phosphatidylglycerophosphate A	767	Δ6 bp						
Cont19	acyl-CoA dehydrogenase	4,225	G→.						
Cont19	acyl-CoA dehydrogenase	4,226	C→.						
Cont67	PhoP/Q and low Mg ²⁺ inducible outer membrane protein H1	857	Δ8 bp						
Cont201	lipopolysaccharide B-band O-antigen biosynthesis protein	29,039	G→.						
Cont58	RecName: Full=Transcriptional activator protein LasR [Pseudomonas aeruginosa PAO1]	29,332	+15 bp						
Cont42	RecName: Full=DNA mismatch repair protein MutS	46,203	+9 bp						
Cont42	spermidine dehydrogenase, SpdH	148,787	+C						

17 **Table S1D. Details of DIP (deletion, insertion and other polymorphisms) mutations detected in *P. aeruginosa* DPB evolved**

18 **populations.** Mutations observed in control populations were removed avoid the effect of long-term growth on LB. Gray shading indicates

19 fixed mutations and gray shading with grid indicates mutations with less than 80% frequency (Breseq consensus mode).

Contig	Annotation	Gene position (Contig)	Mutation in contig	BAC(+)_1	BAC(+)_2	BAC(+)_3	BAC(-)_1	BAC(-)_2	BAC(-)_3
Cont110	PmrA: two-component regulator system response regulator PmrA	94,728	+20 bp						
Cont98	Full=TPR repeat-containing protein PA4667	6,631	Δ 4 bp						
Cont48	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase	164,191	+12bp						
Cont112	histidine kinase	27,735	Δ 9 bp						
Cont112	histidine kinase	27,867	Δ 9 bp						
Cont115	peptidase S41	44,082	G→.						
Cont43	TetR family transcriptional regulator	217,721	+G						
Cont85	Full=Chloramphenicol acetyltransferase; AltName: Full=Xenobiotic acetyltransferase; Short=XAT	22,185	Δ 1,817 bp						
Cont118	O-antigen acetylase	68,790	+G						

Cont118	O-antigen acetylase	70,620	C→.						
Cont92	alpha-galactosidase	14,273	+C						
Cont92	RecName: Full=60 kDa chaperonin; AltName: Full=GroEL protein; AltName: Full=Protein Cpn60	71,372	Δ36 bp						
Cont96	RecName: Full=Cytochrome c551 peroxidase; Short=CCP; Short=Cytochrome c peroxidase; Flags: Precursor	50,169	Δ32 bp						
Cont72	two-component sensor PhoQ	226,316	Δ92 bp						
Cont123	nonCDS	804	219 bp x 2						
Cont55	TonB-dependent receptor	7,536	+GCGCTGG						

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26 **Supplementary Table S1E. Mutations in *pmrB* and *pmrA* genes found in *P. aeruginosa* DP BAC(-) populations (Breseq**
 27 **polymorphism mode).**

Comparison	Contig	Annotation	Gene position (Contig)	Mutation in contig	Amino acid change	frequency
BAC(-)_2_reads_vs_ancestor_contig	Cont53	<i>pmrA</i>	10,036	G→C	L(ancestor and BAC(+)_2) 59 V(BAC(-)_2)	26.80%
		<i>pmrB</i>	8,872	Δ7 bp		7.10%
		<i>pmrB</i>	8,249	C→T	G(ancestor and BAC(+)_2) 421 S(BAC(-)_2)	55.80%
BAC(-)_3_reads_vs_ancestor_contig		<i>pmrB</i>	9,449	A→G	F(ancestor and BAC(+)_3) 21 V(BAC(-)_3)	55.20%