

**SUPPLEMENTARY TABLE 2.**

Gene with mutation	Contig in reference	Locus in reference	Mutational effect	Mutation class	Evolved isolate (condition)	PGD functional classification*	Pathways*
<b>Signal transduction</b>							
<i>bifA</i> cyclic-Di-GMP phosphodiesterase	PAO1_108	PAO1_04439	missense_variant c.1796A>T p.Lys599Met	Non-synonymous; missense	C6a, C9a, C3b, C5b	Cell wall/LPS/capsule; Motility and attachment	Biofilm formation
<i>yfiR</i> protein	PAO1_93	PAO1_03876	missense_variant c.404T>A p.Val135Glu	Non-synonymous; missense	C12a	Cell wall/LPS/capsule	
			missense_variant c.281T>A p.Val94Glu	Non-synonymous; missense	C11a		
<i>wspA</i> probable chemotaxis inducer	PAO1_30	PAO1_01217	missense_variant c.992C>T p.Ser331Leu	Non-synonymous; missense	C1a, C2a, C2b	Motility and attachment; Adaptation, protection; chemotaxis	Two-component system; Chemotactic transducer (MCP); Chemosensory; Biofilm formation
			frameshift_variant c.688delA p.Ile230fs	Deletion	C3a, C4a, C6b, C4b, O3a		
			frameshift_variant c.880_911delACCATCGCCAGGACCAGGCCAGTTGCGCAGT p.Thr294fs	Deletion	C5a		
			frameshift_variant c.445delG p.Ala149fs	Deletion	C1b, C7b		
<i>wspF</i> probable methylesterase	PAO1_30	PAO1_01222	missense_variant c.61G>C p.Ala21Pro	Non-synonymous; missense	O5a, O12a, O7a, O2b	Chemotaxis; transcriptional regulators; motility and attachment	Two-component system; Chemotaxis; Chemosensory; Biofilm formation
			missense_variant c.842C>A p.Ala281Asp	Non-synonymous; missense	O8a		
			stop_gained c.658C>T p.Gln220*	Non-synonymous; stop	O1a		
			conservative_inframe_deletion c.37_63delGTCGAGGCGCTGCGCCGCGCTGGCC p.Val13_Ala21del	Deletion	O2a		
			missense_variant c.821C>T p.Thr274Ile	Non-synonymous; missense	O4a		
			stop_gained c.418C>T p.Gln140*	Non-synonymous; stop	O3b, O1b		
<i>Motility regulator morA</i>	PAO1_108	PAO1_04681	missense_variant c.3464T>A p.Leu1155Gln	Non-synonymous; missense	O11a, O10a, O4b, O5b	Membrane proteins	
<i>Glucose transport sensor gtrS</i>	PAO1_36	PAO1_01743	missense_variant c.1014C>G p.His338Gln	Non-synonymous; missense	O5b	Two-component regulatory systems	Two-component system
<b>Secretion</b>							
<i>tssL1</i> membrane protein	PAO1_11	PAO1_00042	synonymous_variant c.1137G>A p.Pro379Pro	Synonymous	C3b	Hypothetical, unclassified, unknown; Protein	HCP secretion island (HIS-I) type VI secretion system; Bacterial secretion system

							secretion/export apparatus
<b>Translation</b>							
Elongation factor <i>G fusA1</i>	PAO1_21	PAO1_00659	missense_variant c.953C>T p.Ser318Leu	Non-synonymous; missense	C3a	Translation, post-translational modification, degradation	
	PAO1_21	PAO1_00659	missense_variant c.1546G>A p.Gly516Ser	Non-synonymous; missense	C8a, C10b		
<b>Transcription</b>							
Transcriptional regulator <i>mvfR</i>	PAO1_93	PAO1_04000	conservative_inframe_deletion c.109_120delTCGGCGGTCAGC p.Ser37_Ser40del	Deletion	O11a, O10a, O4b, O5b	Transcriptional regulators; Biosynthesis of cofactors, prosthetic groups and carriers	Quorum sensing; biofilm formation
			frameshift_variant c.782_785dupGCGG p.Ile263fs	Duplication	C3b, C5b		
			missense_variant c.101C>T p.Ala34Val	Non-synonymous; missense	O9a		
			missense_variant c.112G>A p.Ala38Thr	Non-synonymous; missense	O1b		
			missense_variant c.440T>C p.Ile147Thr	Non-synonymous; missense	C4a, C4b		
			missense_variant c.527A>C p.His176Pro	Non-synonymous; missense	C6b		
Transcriptional regulator <i>mexT</i>	PAO1_41	PAO1_02477	conservative_inframe_insertion c.389_390insCCT p.Val130_Leu131insLeu	Insertion	C12a, C6a, C2a, C13a, C7a, C5a, C5b, C2b, C9b, C6b, C4b, C1b, C7b, O5a, O7a, O8a, O1a, O2a, O11a, O10a, O4a, O2b, O3b, O1b, O4b	Transcriptional regulators	
Glycerol-3-phosphate regulon repressor <i>glpR</i>	PAO1_30	PAO1_01342	missense_variant c.169G>A p.Ala57Thr	Non-synonymous; missense	C8b	Transcriptional regulators	
Transcriptional regulator <i>lasR</i>	PAO1_93	PAO1_03564	missense_variant c.628T>C p.Phe210Leu	Non-synonymous; missense	C6b	Transcriptional regulators; Adaptation, protection	Quorum sensing; biofilm formation
Transcriptional regulator <i>vfr</i>	PAO1_20	PAO1_00624	frameshift_variant c.594dupG p.Leu200fs	Duplication	O5b	Transcriptional regulators	Two-component system; Quorum sensing; Biofilm formation
<b>Motility</b>							
<i>fimL</i> protein	PAO1_88	PAO1_03161	stop_gained c.1528C>T p.Gln510*	Non-synonymous; stop	O4b	Motility and attachment	
			conservative_inframe_insertion c.1340_1341insCCTGGC p.Gly447_Leu448insLeuAla	Insertion	C5b		

Type 4 fimbrial biogenesis protein <i>pilY1</i>	PAO1_108	PAO1_04632	stop_gained c.2993C>A p.Ser998*	Non-synonymous; stop	C6b	Motility and attachment	Pilin biosynthesis
Type 4 fimbrial biogenesis protein <i>pilM</i>	PAO1_122	PAO1_05151	frameshift_variant c.670delG p.Gly224fs	Deletion	C4b	Motility and attachment	Pilin biosynthesis
Twitching motility protein <i>pilT</i>	PAO1_19	PAO1_00365	conservative_inframe_deletion c.970_984delGTCGCAAGGGCCTG p.Val324_Leu328del	Deletion	O1b	Cell wall/LPS/capsule; Motility and attachment	Pilin biosynthesis
<b>Unknown</b>							
Hypothetical protein	PAO1_79	PAO1_02747	conservative_inframe_deletion c.868_888delTTTGAGACTGCTATTTCCAG p.Phe290_Gln296del	Deletion	O3b		
	PAO1_88	PAO1_03081	missense_variant c.523T>C p.Phe175Leu	Non-synonymous; missense	C9b		
	PAO1_120	PAO1_04769	frameshift_variant c.281dupC p.Leu95fs frameshift_variant c.716delC p.Pro239fs	Duplication Deletion	C2b C1b		
<b>Unknown (phage related)</b>							
Hypothetical protein from bacteriophage Pf1	PAO1_100	PAO1_04289	synonymous_variant c.246G>T p.Gly82Gly	Synonymous	C2b, C1b		
			synonymous_variant c.198T>C p.Ser66Ser	Synonymous	C2b, C1b		

**Footnotes;** PDG, Pseudomonas Genome database; \* Functional classifications and pathways according to the Pseudomonas Genome Database