Supplemental Material

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Table S1. **Genome characteristics of *P. aeruginosa* MPAO1 and PAO1-UW.**

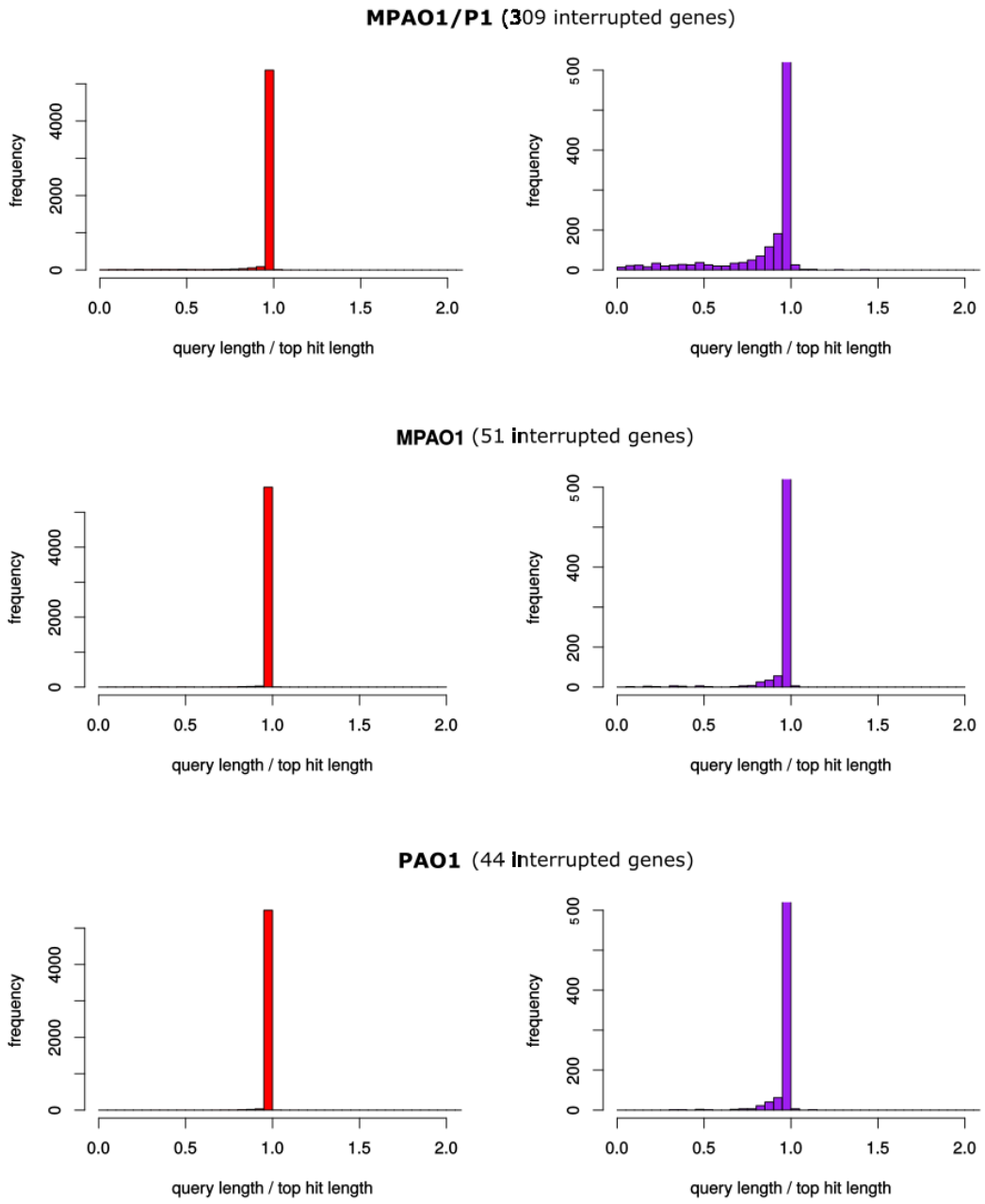
	***P. aeruginosa* MPAO1**	***P. aeruginosa* PAO1-UW***
Genbank accession #	CP027857	NC_002516
No. chromosomes (plasmids)	1 (0)	1 (0)
Size (bp)	6,275,467	6,264,404
G+C content (%)	66.5	66.6
Coverage (PacBio)	180x	n.a.
Coverage (Illumina MiSeq)	101x	n.a.
Total No. of genes	5,926	5,697
No. of protein-coding genes (CDSs)	5,799	5,572
No. of rRNA operons (16S, 23S, 5S)	4,4,4	4,4,4
No. of tRNA genes	63	63
No. of pseudogenes	48	19
No. of ncRNA, tmRNA	4, -	29, 1
No. of 4.5S rRNA	-	1
Prophages	3	2

*We interchangeably use *P. aeruginosa* PAO1 or PAO1-UW.

Table S2. Detailed annotation & integrated information for data mining (Master table).

See separate Excel file. The file contains detailed annotation for all 5,799 annotated MPAO1 CDS. Furthermore, it includes information whether the genes are conserved or unique compared to *P. aeruginosa* PAO1, the respective PAO1 homolog (and gene name, where applicable), the genes missed in three Illumina short read-based assemblies of MPAO1 strains [1] [2], gene essentiality status from our study and previous data sets [3] [4], protein expression evidence from our study (biofilm versus planktonic growth), and additional information about protein domains, families, patterns, signatures, a Gene Ontology (GO) classification, a prediction of subcellular localization, lipoproteins, etc. We did not specifically assess short MPAO1-unique genes, whose gene essentiality status is more difficult to robustly classify. Instead, we added a proteogenomics element to enable identification of novel short proteins (main article).

Figure S1. The genome of strain MPAO1/P1 contains more interrupted genes.



An analysis with Ideel (https://github.com/mw55309/ideel) uncovered large differences of the number of predicted pseudogenes/interrupted genes, which can serve as one parameter to estimate genome completeness. For MPAO1/P1 (5,791 CDS), about six times as many putative pseudogenes/interrupted genes were identified compared to the complete MPAO1 (5,799 CDS) and PAO1-UW (5,572 CDS) genome sequences (MPAO1/P1: 309; MPAO1: 51; PAO1-UW: 44). A complete genome typically shows a narrow peak around 1, i.e., most of the CDS have a full length BlastP hit against the respective UniProt entry, and a shallow tail of the distribution towards the left (see zoomed region in the right plots).

Table S3. Summary of SNP differences between strain MPAO1 and PAO1.

The table (see separate Excel file) lists the differences between our complete genome assembly of MPAO1 and the genome sequence of PAO1-UW [5], the PAO1 type strain. We could confirm the 16 SNPs reported previously for both strains [6], one SNP only in MPAO1 and six SNPs observed as base exchanges in both strains. We could also confirm nine of the SNPs that had been reported for the PAO1 DSM strain only [6] (synonymous substitutions in Phage pf1 protein) which are located at the beginning of the inversion region, one SNP in transcriptional regulator MexT and one intergenic SNP at position 5,033,102 of the MPAO1 genome. In addition, we observed a total of 176 additional SNPs and INDELs between PAO1 and MPAO1 that were not reported by Klockgether and colleagues, as their comparison had focused on selected genomic regions of the two strains [6].

Table S4. **List of shared and specific gene clusters for strains PAO1 and MPAO1.**

Gene clusters specific to either PAO1 (21) or MPAO1 (232), and those shared between the two strains (5,534) as returned from an analysis with Roary [7] are listed in a separate Excel file, along with genomic coordinates, annotation and COG classification. For the MPAO1-specific gene clusters, information about essentiality was computed based on the very important dataset by Lee and colleagues and using the scripts they provided in their Supplementary Material [3].

Table S5. **Gene ontology categories among 232 unique MPAO1 gene clusters.**

GO accession	GO description	Unique genes	total proteins annot.	unique proteins annot.	p-value
Biological Process					
GO:0006468	protein phosphorylation	MPAO1_11765, MPAO1_24875, MPAO1_24880	10	3	2.90E-05
GO:0030153	bacteriocin immunity	MPAO1_05695, MPAO1_20105	5	2	0.00041
GO:0006571	tyrosine biosynthetic process	MPAO1_09400	1	1	0.00664
GO:0006470	protein dephosphorylation	MPAO1_24870	5	1	0.03276
GO:0015074	DNA integration	MPAO1_24800	7	1	0.04557
Molecular Function					
GO:0004672	protein kinase activity	MPAO1_11765, MPAO1_24875, MPAO1_24880	85	3	0.0226
GO:0003866	3-phosphoshikimate 1-carboxyvinyltransferase	MPAO1_09400	1	1	0.0073
GO:0004308	exo-alpha-sialidase activity	MPAO1_11350	1	1	0.0073
GO:0004665	prephenate dehydrogenase (NADP+) activity	MPAO1_09400	1	1	0.0073
GO:0008849	enterochelin esterase activity	MPAO1_13245	1	1	0.0073
GO:0008977	prephenate dehydrogenase (NAD+) activity	MPAO1_09400	1	1	0.0073
GO:0008998	ribonucleoside-triphosphate reductase activity	MPAO1_16050	2	1	0.0145
GO:0004722	protein serine/threonine phosphatase activity	MPAO1_24870	3	1	0.0217
GO:0015643	toxic substance binding	MPAO1_20105	3	1	0.0217
GO:0005102	receptor binding	MPAO1_24810	4	1	0.0288
GO:0016805	dipeptidase activity	MPAO1_06140	4	1	0.0288

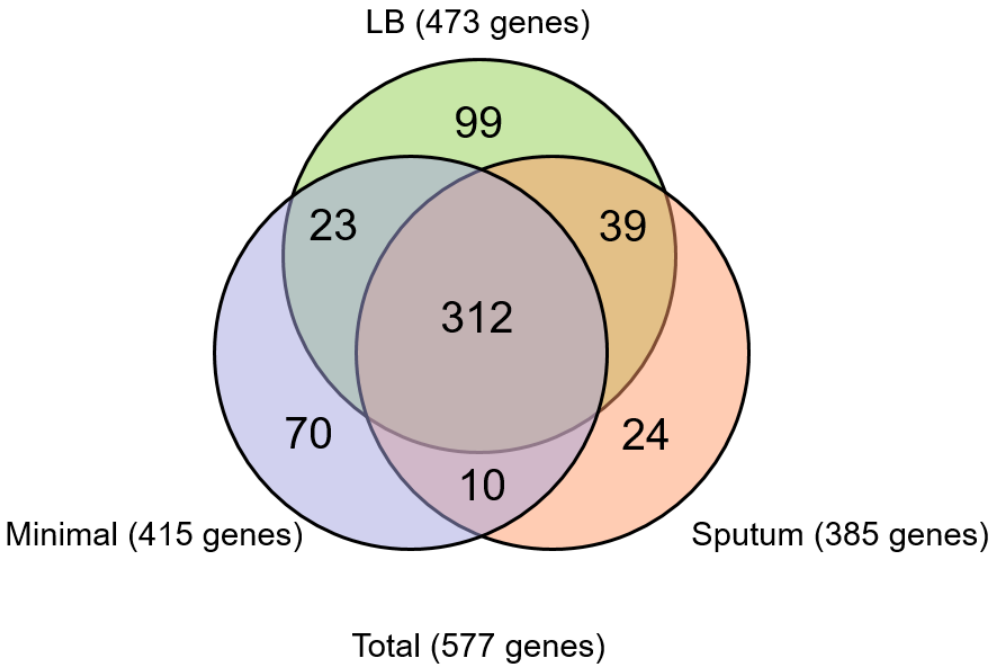
GO categories for which only one gene among the unique MPAO1 genes was affected are shown in gray

Table S6. Re-mapping of Tn-seq data against the MPAO1 genome leads to a higher percentage of mapped reads.

Library Name	# mapped reads		% increase mapped reads	# unique insertion sites		% increase unique insertion sites
	PAO1-UW	MPAO1		PAO1-UW	MPAO1	
LB-1_Rep1	2,095,460	2,099,090	0.17%	92,731	92,938	0.22%
LB-1_Rep2	13,409,799	13,427,972	0.14%	89,678	89,884	0.23%
LB-1_Rep3	8,970,876	8,990,866	0.22%	64,155	64,298	0.22%
LB-2_Rep1	14,495,458	14,546,760	0.35%	82,060	82,239	0.22%
LB-2_Rep2	18,630,794	18,648,695	0.10%	82,441	82,655	0.26%
LB_3	23,922,898	23,952,863	0.13%	110,102	110,331	0.21%
Minimal-1	27,740,278	27,770,384	0.11%	193,648	194,051	0.21%
Minimal-2	13,365,514	13,383,055	0.13%	204,750	205,193	0.22%
Minimal-3	6,441,754	6,449,398	0.12%	250,831	251,570	0.29%
Sputum-1	5,630,125	5,635,321	0.09%	78,706	78,921	0.27%
Sputum-2	7,609,070	7,617,184	0.11%	181,365	181,737	0.21%
Sputum-3	14,491,394	14,508,287	0.12%	48,809	48,930	0.25%
Sputum-4	2,991,136	2,994,255	0.10%	91,856	92,035	0.19%
HumanSerum	754,467	755,143	0.09%	127,543	127,808	0.21%
0.1XLB	8,735,565	8,743,902	0.10%	121,430	121,679	0.21%
BHI	4,438,845	4,443,736	0.11%	174,419	174,796	0.22%

As expected, consistently higher percentages of mapped reads were achieved when mapping the Tn-seq datasets to the complete genome assembly of *P. aeruginosa* strain MPAO1 compared to mapping it to the reference strain PAO1-UW.

Figure S2. Overview of conditionally essential genes in *P. aeruginosa* MPAO1.



Using the scripts released by Lee and colleagues [3], Tn-seq data were re-mapped to our complete MPAO1 genome and compared with data published, i.e., the set of genes essential in either one of the three conditions sputum, minimal medium and LB medium (577 "all essential genes"), as well as the 312 genes essential in all 3 categories ("general essential genes"). This analysis was close to the original results. Due to the higher mapping success of reads to the complete genome sequence, we identified 39 MPAO1-unique "all essential genes" in the MPAO1 genome (Table 2 and Table S5). Overall, 1117 genes were identified as essential in at least one Tn-seq library; thereof, 136 were MPAO1-unique genes (see Table S7). Six of the MPAO1-unique genes were general essential genes.

Table S7. Summary table of 1117 genes essential in at least one Tn-seq library.

See separate Excel file. For completeness, we also show the MPAO1-unique genes that were identified as essential in at least one of the sixteen Tn-seq samples (136; Table S7). The second column shows in how many of the 16 samples a respective gene was called essential. The last three columns indicate the subset of 577 genes essential in at least one of the three primary growth conditions (LB, minimal and sputum), and the subset of 312 genes essential in all three primary growth conditions, i.e., general essential genes (see Methods).

Table S8. Laminar flow conditions achieved in the biofilm chamber.

The flow in the flow chamber had a defined laminar flow. The calculated Reynolds Number (see formulas below table) was 0.103 given a volumetric flow rate 5 µL/min, pressure 0.0108 mbar, the channel specifications given below, and correcting for the viscosity and density of water at 37 °C.

Initial Conditions	
Channel geometry	rectangular
Channel height	200 µm
Channel width	2000 µm
Channel length	30 mm
Fluid viscosity	0.73 cP (Pa.s / 10^3)
Fluid density	0.993 g/cm^3 (kg/m^3 x10^3)
Results	
Pressure	0.0107652 mbar
Flow rate	5 µL/min
Velocity	0.000208333
Reynolds number	0.103051 (**laminar flow**)

$$Re = \frac{d * D * v}{\mu}$$

Re: Reynolds Number
d: Density
v: Flow Velocity
D: Hydraulic diameter
µ: Dynamic Viscosity

$$D = \frac{4A}{P}$$

A: section area

P: wettered perimeter

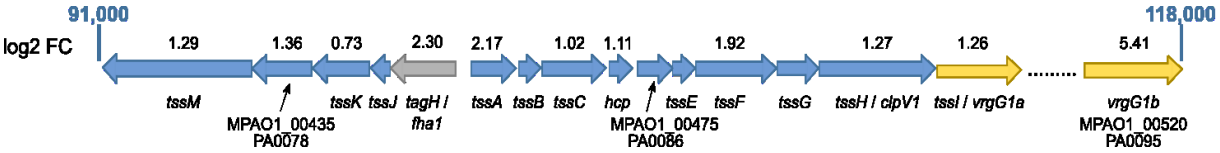
Table S9. List of *P. aeruginosa* MPAO1 mutant strains used in this work.

All mutants are from the UW Genome Center *P. aeruginosa* MPAO1 transposon mutant library (laboratory of Prof. Dr. Colin Manoil). Listed are the name of the mutant strain, the identifier of the respective gene in PAO1-UW and the MPAO1 locus tag, the gene name and a putative function. Note: gene names in the UW library are listed as PA0160-G03::ISphoA/hah (given here for strain PW1274 as one example).

Strain name	PAO1 gene identifier	MPAO1 locus tag	Gene name	Putative function
PW1274	**PA0160**	MPAO1_00860		hypothetical protein
PW7893	**PA0357**	MPAO1_01890	*mutM*	formamidopyrimidine-DNA glycosylase
PW1808	**PA0440**	MPAO1_02325		probable oxidoreductase
PW1871	**PA0476**	MPAO1_02520		probable permease
PW2290	**PA0711**	MPAO1_22480		hypothetical protein
PW2385	**PA0761**	MPAO1_22195	*nadB*	L-aspartate oxidase
PW2642	**PA0898**	MPAO1_21485	*aruD*	succinylglutamate 5-semialdehyde dehydrogenase
PW2661	**PA0914**	MPAO1_21390		hypothetical protein
PW3211	**PA1224**	MPAO1_19730		probable NAD(P)H dehydrogenase
PW3497	**PA1373**	MPAO1_18945	*fabF2*	3-oxoacyl-acyl carrier protein synthase II
PW3660	**PA1467**	MPAO1_18470		hypothetical protein
PW3859	**PA1599**	MPAO1_17765		probable transcriptional regulator
PW3904	**PA1629**	MPAO1_17615		probable enoyl-CoA hydratase/isomerase
PW4005	**PA1693**	MPAO1_17275	*pscR*	translocation protein in type III secretion
PW4095	**PA1755**	MPAO1_16955		hypothetical protein
PW4171	**PA1804**	MPAO1_16665	*hupB*	DNA-binding protein HU
PW4474	**PA1997**	MPAO1_15660		probable AMP-binding enzyme
PW4590	**PA2084**	MPAO1_15180		probable asparagine synthetase
PW4975	**PA2361**	MPAO1_13710		hypothetical protein
PW5552	**PA2716**	MPAO1_11820		probable FMN oxidoreductase
PW5732	**PA2825**	MPAO1_11185		probable transcriptional regulator
PW5923	**PA2928**	MPAO1_10660		hypothetical protein
PW6141	**PA3064**	MPAO1_09940	*pelA*	PelA
PW6275	**PA3137**	MPAO1_09535		probable major facilitator superfamily (MFS) transporter
PW6504	**PA3279**	MPAO1_08785	*oprP*	Phosphate-specific outer membrane porin OprP precursor
PW6719	**PA3391**	MPAO1_08185	*nosR*	regulatory protein NosR
PW6755	**PA3410**	MPAO1_08085		probable sigma-70 factor, ECF subfamily
PW6868	**PA3470**	MPAO1_07770		hypothetical protein
PW6985	**PA3534**	MPAO1_07440		probable oxidoreductase

PW7021	**PA3552**	MPAO1_07345	*arnB*	ArnB
PW7067	**PA3574**	MPAO1_07230		probable transcriptional regulator
PW7383	**PA3772**	MPAO1_06190		hypothetical protein
PW7566	**PA3890**	MPAO1_05560		probable permease of ABC transporter
PW8169	**PA4224**	MPAO1_03835	*pchG*	pyochelin biosynthetic protein PchG
PW8212	**PA4282**	MPAO1_22735		probable exonuclease
PW8322	**PA4338**	MPAO1_23025		hypothetical protein
PW8365	**PA4362**	MPAO1_23150		hypothetical protein
PW8707	**PA4578**	MPAO1_24275		hypothetical protein
PW8936	**PA4711**	MPAO1_25105		hypothetical protein
PW8965	**PA4726**	MPAO1_25185	*cbrB*	two-component response regulator CbrB
PW9431	**PA5020**	MPAO1_26730		probable acyl-CoA dehydrogenase
PW9793	**PA5219**	MPAO1_27780		hypothetical protein
PW9856	**PA5261**	MPAO1_28000	*algR*	alginate biosynthesis regulatory protein AlgR
PW9891	**PA5281**	MPAO1_28105		probable hydrolase
PW9895	**PA5283**	MPAO1_28115		probable transcriptional regulator
PW9934	**PA5304**	MPAO1_28225	*dadA*	D-amino acid dehydrogenase, small subunit
PW10082	**PA5384**	MPAO1_28660		probable lipolytic enzyme
PW10195	**PA5442**	MPAO1_28970		conserved hypothetical protein
PW10219	**PA5455**	MPAO1_29035		hypothetical protein

Figure S3. Several members of the H1-T6SS are upregulated in biofilm.



Genomic region of *P. aeruginosa* MPAO1 that shows part of the H1-T6SS region (roughly from nucleotide 91,000-118,000); gene names or MPAO1 locus tags are shown (with the respective PAO1 homolog below). The colors were selected as in [8] and indicate structural elements (blue), vgrGs (yellow) and other known T6SS genes (gray). The shotgun proteomics data (log2 fold change biofilm over planktonic is shown above the arrows) indicated that several (9 of 14, 64%) of the proteins encoded by structural elements of the H1-T6SS and secreted proteins [9] were upregulated in biofilm cells compared to planktonic cells. The H1-T6SS has been described as “a molecular gun firing toxins (Tse1-Tse7)” and has been implied “to challenge the survival of other bacteria and help *P. aeruginosa* prevail in specific niches” [8]. This specific H1-T6SS has recently been shown to be highly relevant for the ability of *P. aeruginosa* strains to dominate in multi-species biofilms [10]. Notably, all three VgrG proteins that are co-regulated with this T6SS (VgrG1a-c) were upregulated, including VGR1c (MPAO1_11985; PA2685; 2.53 log2 FC). In contrast, none of the seven other members of the VgrG family (total of 10) [9] was expressed (see Supplementary **Table 2**).

Our pilot study proteomics dataset covered about 33% of the annotated MPAO1 proteins. This coverage was below that of the extensive proteomics dataset that had allowed to uncover expression evidence for all *Bartonella henseale* Type IV secretion system (T4SS) members [11]. However, that coverage was only be achieved by employing several elaborate fractionation and enrichment strategies, which was beyond the scope of this pilot study. Several of the structural members of the H1-T6SS include shorter proteins and membrane proteins, both of which are more difficult to detect by shotgun proteomics.

Table S10. Summary of annotation clusters created for the MPAO1 iPtgxDB.

Annotation source (tag in identifier)	Anno-tations	Clusters*	New clusters	New reductions	New extensions	Total clusters	Total ids
RefSeq (refseq)	5,851	5,851	5,851	0	0	5,851	5,851
Prodigal (prod)	5,691	5,691	55	309	150	5,906	6,365
ChemGenome (chemg)	4,616	4,616	1,703	81	1,745	7,609	9,894
In silico ORF (orf)	155,710	70,655	63,065	78	76,881	70,674	149,918

* See the original paper for a detailed description of the annotation clusters [12] or also the website https://iptgxdb.expasy.org/creating_iptgxdbs/ for more information.

File S11. Design of the microfluidic chamber as a CAD file.

See separate DWG file.

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