# Phenotypic characterisation of an international *Pseudomonas aeruginosa* reference panel: Strains of cystic fibrosis origin show less *in vivo* virulence than non-CF strains

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**Abstract:**

*Pseudomonas aeruginosa* causes chronic lung infections in people with cystic fibrosis (CF) and acute opportunistic infections in people without CF. Forty two *P. aeruginosa* strains from a range of clinical and environmental sources were collated into a single reference strain panel to harmonise research on this diverse opportunistic pathogen. To facilitate further harmonized and comparable research on *P. aeruginosa*, we characterised the panel strains for growth rates, motility, virulence in the *Galleria mellonella* infection model, pyocyanin and alginate production, mucoid phenotype, lipopolysaccharide (LPS) pattern, biofilm formation, urease activity, antimicrobial and phage susceptibilities. Phenotypic diversity across the *P. aeruginosa* panel was apparent for all phenotypes examined agreeing with the marked variability seen in this species. However, except for growth rate, the phenotypic diversity among strains from CF versus non-CF sources was comparable. CF strains were less virulent in the *G. mellonella* model than non-CF strains (p=0.037). Transmissible CF strains generally lacked O antigen, produced less pyocyanin, and had low virulence in *G. mellonella*. Further, in the three sets of sequential CF strains, virulence, O-antigen expression and pyocyanin production were higher in the earlier isolate compared to the isolate obtained later in infection. Overall, full phenotypic characterization of the defined panel of *P. aeruginosa* strains increases our understanding of the virulence and pathogenesis of *P. aeruginosa* and provides a valuable resource for the testing of novel therapies against this problematic pathogen.

## INTRODUCTION

*Pseudomonas aeruginosa* is a Gram-negative bacterium that causes opportunistic infections including burn wound infections, urinary tract infections, keratitis, otitis externa and respiratory tract infections in susceptible individuals. *P. aeruginosa* is a major pulmonary pathogen in people with cystic fibrosis (CF), contributing significantly to their observed decline in lung function and morbidity, with over 50% of people with CF being chronically colonised by adulthood. Most of the extensive research carried out on *P. aeruginosa* has focussed on selected strains (e.g. PAO1 or PA14), several of which are genotypically distinct from the more abundant clinical strains (De Soyza *et al.*, 2013). It has been widely demonstrated that *P. aeruginosa* is a highly diverse species (Fothergill *et al.*, 2010; Mowat *et al.*, 2011). Given the diversity and adaptability of *P. aeruginosa* in a broad range of environments, including non-clinical settings such as soil, lake water and plants, research focusing on a single or very small number of strains may lead to conclusions that are not relevant to the clinical scenario or the infection being examined. To address this, we collated an international *P. aeruginosa* reference panel allowing better consolidation of research into the pathogenesis of this organism (De Soyza *et al.*, 2013). Certain *P. aeruginosa* strains from CF patients are genotypically indistinguishable from environmental strains despite the extensive differences in habitats (Wiehlmann *et al.*, 2007). Therefore, the panel comprised 42 strains that were selected to represent the diversity across *P. aeruginosa* and included strains from a wide variety of clinical and environmental sources. In addition, strains from a range of geographical regions, transmissible (epidemic) CF strains and representatives of sequential strains from early to late (i.e. chronic) CF infection were incorporated into the panel (De Soyza *et al.*, 2013).

Several panel strains have been studied previously and specific virulence properties and other traits have been examined in individual strains or compared across small groups of strains. Here, we report the standardised phenotypic characterisation of the panel, including growth characteristics, motility, virulence in *Galleria mellonella*, production of alginate and virulence factors, including pyocyanin and LPS; urease activity, biofilm formation, quorum sensing (QS), antibiotic resistance and phage susceptibility. Our aims were to facilitate the future use of this panel for broad comparisons across a wide range of phenotypes and to compare the strains of CF and non-CF origin for phenotypic differences that depend on their particular niches.

## METHODS

**Growth conditions.** The strain panel is available from the BCCM/LMG Bacteria Collection, Gent, Belgium, (http://bccm.belspo.be/about/lmg.php) (Table 1) (De Soyza *et al.*, 2013). The original panel comprised of 43 strains; however, strain NN2 was withdrawn from the BCCM collection and excluded from this study due to inconsistencies in its taxonomic identity when it was shared across the multiple laboratories who participated in the current research. Strains were routinely grown on Tryptone Soya Agar (TSA; Oxoid Ltd., UK) overnight (16-18 h) at 37°C. Overnight broth cultures were prepared by inoculating 3 ml Luria Bertani broth (LB; Oxoid) with fresh growth from a pure streak plate. Cultures were grown for 16-18 h at 37°C, shaking at 150 rpm.

**Growth curve analysis.** Growth was examined on a Bioscreen C instrument (Labsystems, Finland) in 200 µl Mueller-Hinton broth (MHB) inoculated with approximately $10^5$ cfu $ml^{-1}$. Growth in liquid culture was monitored for 48 h at 37°C and turbidity measurements were taken every 15 min after shaking the microplates for 10 sec. A scatterplot was used to visualise growth curves and the growth parameters were analysed with the grofit package (Kahm *et al.*, 2010) using R statistical software (R-Core-Team, 2013). Strains producing growth curves which could not be modelled accurately by grofit (discordance between model and model-free-spline fits) were excluded. The distribution of growth parameter data was examined with BoxPlotR (Michaela *et al.*, 2014).

**Virulence in the *G. mellonella* insect infection model.** Virulence was determined according to published methods (Lore *et al.*, 2012), with some modifications. Wax moth larvae (Livefoods Direct, Sheffield, UK) were stored at 15°C and used within 4 weeks. Overnight bacterial cultures in LB were diluted 1:10 and grown to an $OD_{600nm}$ of 0.4-0.8. Cultures were centrifuged and bacterial cells resuspended in 10 mM $MgSO_4$ (Sigma-Aldrich) and serially diluted to $10^{-9}$. Each dilution was injected (10 µl) into the hindmost proleg of healthy larvae (6 per group). The same volume of $MgSO_4$ was injected into one group as a control. To preserve the mucoid phenotype of IST27, it and its non-mucoid revertant were serially diluted directly from *Pseudomonas* Isolation Agar (PIA) plates prior to infection. Bioburden was determined by plating 10 µl of each dilution onto LB agar and colonies counted after 24 h. Injected larvae were incubated at 37°C for 24 h and $LD_{50}$ values determined using log graph paper.

**Motility assays.** Agar concentrations were prepared by adding molecular biology grade agarose (Severn Biotech Ltd., UK) to either LB or Basal Salts Medium supplemented with 0.4% (w/v) glucose (BSM-G) and all agar plates were poured on an even surface. At least two biological replicates per

strain were performed for each assay (Rashid & Kornberg, 2000). Swimming motility was assessed by inoculating the surface of a 0.3% (w/v) LB agar plate with overnight culture using a sterile toothpick. Swimming plates were incubated overnight at 37°C for 16-18 hours. Media used to assess swarming motility were 0.5% (w/v) LB agar and BSM-G agar. Swarming plates were surface inoculated with growth from an overnight culture using a sterile toothpick, incubated at 30°C for 16-18 h. The diameters of the swimming and swarming zones were calculated by taking an average of two perpendicular measurements. Strains were scored as non-motile (diameter ≤ 5 mm), motile (diameter > 5 mm and ≤ 60 mm), or highly motile (diameter > 60 mm) in swimming and swarming assays.

Twitching motility was assessed using 1% (w/v) LB agar. Twitch plates were stab inoculated to the base of the petri dish with an overnight culture. Following incubation for 16-18 h at 37°C, twitch plates were dried and agar removed before zones of motility at the agar/petri dish interface were stained with 0.5% (w/v) Coomassie brilliant blue R250 (Sigma-Aldrich) for 2 min (McMichael, 1992). After removal of excess stain, diameter of twitching zones was calculated by taking an average of two perpendicular measurements. Strains were scored as non-motile (diameter ≤ 5 mm), motile (diameter > 5 mm and ≤ 30 mm), or highly motile (diameter > 30 mm).

**Auxotrophy.** Approximately 50,000 cells were spotted on either Mueller Hinton Agar (MHA) or Davis Minimal Agar (DMA). The strains that grew on both media were considered prototrophs, whereas those growing on MHA but not on DMA were considered auxotrophs and subjected to further analysis. Identification of specific amino acid requirements was done using DMA supplemented with: 1) single or multiple amino acids at concentrations of 20 μg $ml^{-1}$; or 2) combinations of 19 amino acids at 20 μg $ml^{-1}$ (Fluka, Switzerland) (Barth & Pitt, 1995). Control agar, such as MHA and DMA without amino acids, was included in all tests; plates were incubated at 37$^{o}$C for 48 h.

**Production of N-acyl homoserine lactones (AHLs).** AHL production was determined using two biosensor strains (*Escherichia coli* JB523 and *P. aeruginosa* QSIS2) (Brackman *et al.*, 2009). Supernatants of 24-h *P. aeruginosa* cultures grown in MHB at 37°C were added to the biosensor strains and fluorescence (JB523 assay, $\lambda_{ex}$ 475 nm; $\lambda_{em}$ 515 nm) or absorbance (QSIS2 assay) was measured using an Envision Xcite multilabel platereader (Perkin Elmer). For the JB523 assay, which produces green fluorescent protein [GFP] in response to the presence of QS molecules, data were presented as background corrected fluorescence values. These values were obtained by subtracting the fluorescence measured in the sensor strain to which uninoculated MHB was added from the

value measured for each strain. This biosensor is most sensitive to 3-oxo-C6-homoserine lactone (HSL), C6-HSL and 3-oxo-C8 HSL. In the QSIS2 assay, growth was repressed by the presence of QS molecules (mainly 3-oxo-C12 HSL). For this assay, the control to which 200 nM of 3-oxo-C12-HSL and C4-HSL (each) was added was set as "100% QS" and the control to which no N-acyl-HSL or supernatant was added, was set as "0% QS".

**Biofilm formation.** Each strain was loop-inoculated from agar slants into LB (5 ml) and cultured overnight. Aliquots were diluted 1:100 in 10 ml of either MHB, LB or glucose-supplemented M63 medium (M63) composed of 0.02 M $KH_2PO_4$, 0.04 M $K_2HPO_4$, 0.02 M $(NH_4)_2SO_4$, 0.1 mM $MgSO_4$ and 0.04 M glucose. After mixing, 150 µl of bacterial suspension were inoculated into 96-well plates, 10 wells per plate, per strain and incubated 37$^{o}$C for 24, 48, or 72 h. Media were aspirated and the wells washed thrice with PBS before staining with 0.1% crystal violet (CV) after which time the wells were washed with PBS until washes were clear. The CV was solubilized in 70% ethanol and absorbance measured at 570 nm.

**Pyocyanin production.** Pyocyanin was determined as previously described (Essar *et al.*, 1990) using PB medium (Bactopeptone, MgCl2, K2SO4) to maximize pyocyanin production in liquid culture, chloroform for extraction and subsequently HCl for re-extraction. The absorbance of the final solution was measured at 520 nm.

**Phage typing.** Eight strictly lytic *P. aeruginosa* bacteriophages with sequenced genomes (Table S1) were used: LUZ7, LUZ19, LBL3, and ϕKZ, from the collection of the Laboratory of Gene Technology, KU Leuven, Belgium (Ceyssens & Lavigne, 2010), and newly isolated phages KT28, KTN6, KTN4, and PA5oct from the Institute of Genetics and Microbiology collection, University of Wroclaw, Poland. Phage typing was performed following previously published methods (Adams, 1959; Kutter, 2009). Prior to phage sensitivity testing, bacteria were subcultured in TSB (Becton Dickinson, Cockeysville, MD) for 4 to 6 h. To determine bacterial susceptibility to phage-mediated lysis, bacteria grown on liquid TSB medium were transferred directly onto TSA. After drying, 10 µl of phage suspensions ($10^4$ PFU $ml^{-1}$) were applied and incubated at 37$^{o}$C. The plates were checked for the presence of bacterial lysis.

**Antibiotic resistance.** Minimum inhibitory concentrations were performed independently in two separate laboratories using two broth dilution methods, a commercial antibiotic panel TRIOS (Prague, Czech Republic), and Trek Diagnostics Sensititre (UK). Results were read at 24 and 48 h. In

the instances where there was complete disagreement between these methods, E test MIC's BioMérieux (UK) were also performed. All results were interpreted following EUCAST guidelines (EUCAST, version 3.1, 11.2.2013; http://www.eucast.org/clinical_breakpoints).

**Quantification of urease activity.** Individual strains were cultured overnight in Christensen broth at 37 °C with shaking. Fresh Christensen broth was inoculated at a ratio of 1:100 (v/v) with overnight culture and re-cultured as above. Cultures were centrifuged (3000 g, 15 min) and the absorbance of cell supernatants at 560 nm measured in triplicate.

**LPS extraction and analysis.** Overnight cultures were adjusted to an $OD_{600}$ of 2.0 in PBS (1 ml). Cells were lysed in 2% SDS, 4% β-mercaptoethanol and Tris (pH 6.8) and boiled for 10 min. The lysates were treated with proteinase K at 60$^{o}$C for 2 h and stored at -20$^{o}$C. LPS was resolved by electrophoresis on 14% polyacrylamide/Glycine-SDS gels (Lesse *et al.*, 1990; Schagger & von Jagow, 1987) and visualised by silver staining (Marolda *et al.*, 1990).

**Analysis of alginate production.** Overnight cultures inoculated with single colonies of either the characteristic mucoid or nonmucoid phenotype, observed following 24-48 h of growth on PIA plates at 37$^{o}$C, were adjusted to an $OD_{600}$ of 2.0 in PBS. Alginate production was determined by ethanol precipitation of the exopolysaccharide from cell-free supernatants and quantification of uronic acids by the modified carbazole method using sodium alginate from *Laminaria hyperborean* as standard (BDH Chemicals Ltd., Poole, England) (Knutson & Jeanes, 1968). Alginate production based on bacterial growth on solid medium was also assessed using LB and PIA. Plates were inoculated with 100 µl of a cell suspension harvested during the exponential phase of growth and resuspended to obtain a standardized $OD_{640}$ of 0.2 $\pm$ 0.02. After growth for 24 h at 37$^{o}$C, cultures were scraped from the plates, resuspended in 0.9% NaCl and harvested at 20,000 g for 10 min and supernatants used for alginate quantification.

**Statistical analysis.** Unless otherwise indicated all statistical analyses were performed using Minitab statistical software package (v15). The distribution of each quantifiable phenotype was determined by plotting the mean data against the frequency in histograms. To confirm the data distribution observed, an Anderson Darling Test for Normality was performed ($p<0.05$, considered non-normal). Virulence, swimming, swarming on LB and BSM-G agar, AHL, alginate and pyocyanin production, growth rate and biofilm formation on MH, LB and M63 media were all found to have non-normal data distribution. Twitching motility and lag-phase phenotypes followed a normal data

distribution. In addition, the effect of source of the strain (i.e. CF versus non-CF), on phenotype was determined using a general linear model for normally distributed data and Kruskal-Wallis analysis for non-normal distribution. A total of 25 strains were in the CF group and 17 in the non-CF group, including both environmental and non-CF clinical strains. To determine if strain source had an effect on mean data variability, a test for equal variances was carried out by the F-test for normally distributed data and by Levene's test in the cases of non-normally distributed data.

# RESULTS

## Growth curve analysis

The growth curves (Fig. S1) revealed considerable variability in the growth dynamics of each strain. The majority of the strains reached their maximum optical density by 15 h and could be split into two groups: one reaching a higher optical density (> 0.25) and the other reaching a lower density (< 0.20) (Table 1). This has been illustrated in greater detail in the boxplot in Fig. 1(a), which resolved the maximum culture density data for the panel strains into two broad groups. According to this analysis, only one strain (AMT 0023-34) was classified as having an intermediate maximum culture density at 15 h, while the others fell into high and low culture density groups. The widely studied PAO1 strain reached the highest culture density within 15 h (Fig 1(a)).

The grofit package for R statistical software was used to better model growth curve parameters (length of lag phase, maximum growth rate and maximum culture density reached), up to 30 h growth. Most of the transmissible strains (LES B58, LES 400, LES 431 and C3719), in addition to NH57388A and Mil 162, had growth curves that could not be modelled accurately by grofit, possibly because of their long lag phase and/or poorly defined exponential phase. Growth parameters for the remaining 36 strains are displayed in Table 2S and boxplots summarise the spread of the data (Fig. 1(b)). Outliers indicated in the boxplots are strain 968333S, which had a longer lag phase (8.8 h) than the maximum value for all strains (upper whisker = 8.0 hours); 2192 and AMT 0023-34. The latter two strains had longer lag phases (8.0 and 7.9 h, respectively) than the maximum value for CF strains (upper whisker = 7.48). In addition, strains 968333S, AUS52, AUS23 and UCBPP-PA14 reached a lower maximum OD (0.13, 0.16, 0.19 and 0.26, respectively) than the minimum value for all strains (lower whisker = 0.29).

## Autotrophy analysis

Only four auxotrophs were identified in the panel. The CF strain, AUS23 and bronchiectasis strain 968333S required histidine, while the transmissible strain LES B58 required methionine and CF strain NH57388A required both isoleucine and valine. In addition, the transmissible strain LES 400 was an apparent prototroph as it grew weakly on minimal medium, but grew abundantly in the presence of threonine.

**Virulence in the *G. mellonella* model**

The majority of strains were virulent in *G. mellonella* with 31 strains showing LD50 values at 24 h of less than 5 CFU (Table 1). Virulence was non-normally distributed (Anderson-Darling $p<0.005$). When strains that were isolated from CF sources were compared with those that were isolated from other sources, the most striking outcome was that CF strains were significantly less virulent in this acute infection model than strains that were not of CF origin (Kruskal-Wallis, $p=0.037$) (Fig 2). Furthermore, the CF transmissible strains showed considerably low virulence in *G. mellonella,* with six of the eight transmissible strains showing LD50s greater than 650 CFU. Four of these strains (LES B58, LES400, LES431 and C3719) were also those that had long lag phase or poorly defined exponential phases. In contrast, transmissible strains DK2 and AUS23 showed LD50 values ranging between 0.5-2 CFU. The earliest identified LES strain (LES B58) was the most virulent of the three LES strains (LES B58, LES 400 and LES 431). The sequential CF strains (AA2, AA43 and AA44; AMT0023-30 and AMT0023-34; AMT0060-1, AMT0060-2 and AMT0060-3) showed a reduction in virulence over time of chronic infection (Fig 2), consistent with previous studies regarding niche adaptation of *P. aeruginosa* to the CF lung environment (Bragonzi *et al.*, 2009; Lore *et al.*, 2012).

**Motility of *P. aeruginosa* strains**

Motility was variable across the panel with the majority of strains (n=38) displaying at least one form of motility (Table 1, Fig S2). The only observable general trend identified was a higher proportion of CF strains were non-motile compared to those from non-CF sources. Four of the five non-motile strains (LES 400, LES 431, C3719 and AUS52), and four of the five strains capable of only swimming (LES B58, AES-1R, AMT 0023-34 and AMT 0060-2) were CF strains. Furthermore, two of the three sets of sequential strains showed a loss in motility over time of colonisation; strain AMT 0060-3 showed more swarming, swimming and twitching motilities relative to the later strain, AMT 0060-02; while AMT 0023-30 showing more swarming and twitching motility relative to the later strain, AMT 0023-34. Only two strains (IST 27 mucoid and IST 27N) demonstrated 'true' swarming, indicated by the formation of finger-like projections radiating from the inoculation point (Fig. S2).

**AHL production**

AHL production was assessed using two biosensors with different sensitivities for various types of AHLs (Fig. 3). Given that they are biosensors and are based on entirely different principles, they inherently show some variation. Although a direct comparison of results obtained with the two sensors used is difficult, some general trends emerge. Under the conditions tested, the panel shows wide variation in the AHL levels produced. The supernatants of some strains showed very low

signals in both assays (e.g. C3719, AUS23, LMG 14084), while other strains were identified as producers of high levels of AHLs by both systems (AMT 0060-3, IST 27 mucoid, IST 27N, 679, Pr335, U018a, 15108/1 and TBCF10839). Strain 679 (isolated from urine) produced the highest levels as determined by the QSIS2 assay, while RP1 (a CF strain) produced the highest levels in the JB523 assay. There was no correlation between AHL levels and the source of the strains. AHL levels did not correlate with time of colonization in sequential CF strains, for example the early strain AA2 and the late strain AA43 both gave low signals in the QSIS2 assay, while the signal from another late strain (AA44) was much higher.

**Biofilm formation.**

Biofilm growth was compared in three media at three time points. All strains formed biofilm to various degrees depending on medium and time (Table S3). Overall, the majority of strains were generally good biofilm producers, including the majority of CF strains (Fig. 4). There was no correlation between biofilm formation at 48 h and the source of the strains (CF v's non-CF), nor were there any apparent differences in diversity between these two groups (Levene's test, p=0.102). The strains could be divided into poor biofilm-formers ($A_{570}$ < 0.350), intermediate biofilm-formers ($A_{570}$ 0.350 to 0.950) and extensive biofilm-formers ($A_{570}$ > 0.950). The only weak biofilm former among the CF strains was LES 431, which agrees with a previous report (Carter *et al.*, 2010). Of the other CF strains, seven were intermediate biofilm formers, and the rest were extensive biofilm formers. The weakest biofilm-former among the non-CF strains was the water isolate, LMG 14084.

Time was an important parameter: rapid biofilm formers tend to detach over time, while slow biofilm formers steadily increased biofilm biomass until day 3 (Table S3). Therefore comparisons between the biofilm formation capacities of strains should take into account the strain-specific kinetics of biofilm formation. Strains with lower biofilm formation generally produced low or undetectable levels of AHLs, e.g. LMG14084, MI162, NH57388A, AA43, AES-1R, LES431, LES400, PR335, LES B58). Further, strong biofilms were obtained for strains with relatively higher AHL levels (e.g. IST27N, 968333S, AA44, KK1). However, this correlation was not always apparent, e.g. CHA (strong biofilm, low AHL), AUS52 (strong biofilm, no AHL) and PA679 (moderate biofilm, high AHL), indicating that other factors, in addition to AHL production or the presence of an active QS system, play a role in biofilm formation of *P. aeruginosa* under these conditions.

**Pyocyanin production**

Pyocyanin is a major virulence factor of *P. aeruginosa* (Dietrich *et al.*, 2006) and its production was variable across the panel (Table 1). Low pyocyanin was observed in 22 strains ($A_{520}$ < 0.1) with 14

strains producing very low levels ($A_{520}$< 0.05). In contrast, nine strains showed comparatively high levels of pyocyanin ($A_{520}$ > 0.3) (Fig. 5). The pyocyanin levels in the sequential CF strains were lower in the later strain than in the early strain, suggesting that *P. aeruginosa* down-regulates pyocyanin production over time during chronic infection. Most of CF transmissible strains (excluding LES B58 and LES 431) produced negligible amounts of pyocyanin. The serotype 1 strains (Pr335, U018a, CPHL 9433 and 39177) showed very high levels of pyocyanin. There were no significant differences in pyocyanin between CF and non-CF strains as determined by Kruskal-Wallis test (p=0.220), and both CF and non-CF populations showed comparable variation in levels of pyocyanin production (Levene's test, p=0.237). There was no clear correlation between pyocyanin and AHL levels, even though pyocyanin is considered to be QS-regulated. Although many strains with very low levels of pyocyanin production only produced low or undetectable levels of AHL and several strains that produced higher levels of pyocyanin produced moderate to high levels of AHL , there were several strains that showed high pyocyanin levels but with low AHL levels (AA2, LMG14084 and 1709-12) and five that produced low pyocyanin, despite high AHL levels (PA968333S, IST27-N, RP1, KK1, AA44).

**LPS characterisation**

LPS is a major virulence factor in *P. aeruginosa* and consists of lipid A, core oligosaccharide and the highly variable long-chain O-polysaccharide (O-antigen) (Kocincova & Lam, 2011). Some *P. aeruginosa* strains, including PAO1, simultaneously produce two types of structurally and serologically distinct O-antigens in the same cell (A- and B- bands). The A-band is homopolymeric, while the B-band is heteropolymeric and responsible for serotype specificity. In this study, the two O-antigen bands of PAO1 were detected (Fig. 6), while another commonly used strain UCBPP-PA14 lacks the O-antigen the A-band, as previously described (Coulon *et al.*, 2010). PAK and CHA also showed an LPS pattern similar to that previously described (Bezuidt *et al.*, 2013; Rahim *et al.*, 2000).

During CF chronic infections, the O-antigen portion of LPS is often lost (Smith *et al.*, 2006). Indeed, strains LES B58, LES 400 and LES 431, recovered during CF infections, did not exhibit O-antigen, which is in agreement with the previous hypothesis that these strains contain rough LPS (Winstanley *et al.*, 2009). Two other transmissible strains also lacked O-antigen, C3719 and DK2, in contrast to AES-1R and AUS23, also transmissible strains (Fig. 6). The LPS of the sequential strains AA2 (early isolate), AA43 and AA44 (late isolates) is complete and shows an identical O-antigen repeating unit in all three strains, as previously reported (Cigana *et al.*, 2009). It is possible to distinguish the presence of a B-band in AA2 and AA43, but not in AA44. The late strain AMT 0023-34 lost O-antigen production compared to the initial strain AMT 0023-30 (Fig. 6). Strains Pr335, U018a,

CPHL 9433 and 39177 belong to serotype O1 (Pirnay *et al.*, 2009; Stewart *et al.*, 2011) but show a slightly different O-antigen banding pattern in the present study (Fig. 6). The strain Jpn1563 has been described as non-typeable (Pirnay *et al.*, 2009) and lacks B-band, which is used as the basis of *P. aeruginosa* serotyping.

The LPS of strain 39016 showed a complete structure, with the production of both A- and B-bands, but the pattern is distinct from that of PAO1. Another abundant strain, RP1, showed a B-band (Fig. 6). In contrast, the common non-CF strains, 15108/1, 57P31PA showed A- and B- bands while strain 13121/1, showed B-bands only. Two other non-CF strains, 96833S and 679 showed no O-antigen. Strain 2192, recovered from a chronically infected patient in Boston has been previously described as devoid of LPS O-side chains (Mathee *et al.*, 2008); however, we observed low level production of O-antigen side chains (Fig. 6). The genome of the CF strain TBCF10839 has been recently sequenced and harbors a serotype O4-like LPS biosynthesis gene cluster (Klockgether *et al.*, 2013). Accordingly, LPS recovered from this strain shows a complete molecule, including O-antigen A- and B-bands. The clinical CF strains IST27 and IST27N have a similar LPS molecule. LPS of these strains as well as of other clinical strains, C3719, DK2, AUS 52, AES-1R, AUS 23, RP1, 15108/1, 57P31PA, 13121/1, KK1, A5803, 968333S and 679 have not been previously reported.

**Alginate production**

Strains AMT 0060-2, CHA, IST 27, 968333S and 2192 produced the highest levels of alginate (Table 1, Fig S3(a)), consistent with the previous observations of mucoidy for these strains (Leitao *et al.*, 1996; Pier *et al.*, 1983; Toussaint *et al.*, 1993). However, a few strains reported as being mucoid (AA43 and NH57388A) or having upregulated alginate production (LES400) (Bragonzi *et al.*, 2006; Hoffmann *et al.*, 2005; Salunkhe *et al.*, 2005), showed low levels of alginate. Since growth of these strains on PIA plates did not generate mucoid colonies, it is likely that reversion of the mucoid phenotype has occurred upon subculturing. Given that growth conditions affect stability of the mucoid phenotype and alginate production, the alginate production of IST 27 and three other strains grown on either LB or PIA plates was compared. The mucoid strains AMT 0060-2 and IST27 revealed increased alginate production on PIA plates, while the non-mucoid strains produced low alginate amounts on PIA (Fig. S3(b)). This phenotype correlated with virulence in *G. mellonella;* overnight cultures of IST27 mucoid and IST27N strains in LB showed comparable high virulence levels (LD50s of 0.37 and 0.36 for IST27 and IST27N, respectively), while infection of the larvae with mucoid IST 27 from a PIA plate showed a substantial reduction in virulence (mean LD50 of 412 CFU), while the virulence of the non-mucoid strain was comparable with that observed when liquid broth was used (LD50 = 0.98).

**Phage Typing**

Phage typing was carried out using well characterized, genome sequenced, bacteriophages belonging to various genus and with high lytic potency. Two phages represented small podoviruses (LUZ7, LUZ19), three medium size myoviruses were from the *Pbunalikevirus* genus (LBL3, KT28, KTN6) and three belonged to giant myoviruses (ϕKZ, KTN4, PA5oct) were used (Table S1). Phages LUZ19, ϕKZ, KTN4 and PA5oct require type IV pili for host infection; LUZ7, KT28, KTN6 are LPS-dependent, whereas LBL3 cannot infect either wild type or the PAO1 mutants (Drulis-Kawa and colleagues, unpublished).

All eight phages combined infected 86% of tested *P. aeruginosa* strains (Table 2). Single phages from the collection were sufficiently potent to propagate 23-46% of *P. aeruginosa* panel strains, regardless of geographic and infection origin. The giant ϕKZ phage was the most potent, whereas the giant PA5oct infected only 10 strains. Three CF strains (2192, 1709-12, RP1) and two non-CF strains (39016, 39177) were resistant to phage infection, while other three were only intermediate susceptible to LBL3 phage activity (all epidemic LES strains). The analysis of phage typing in sequential strains based on phage receptor specificity gave varying results. The early CF strain, AA2, showed lower susceptibility to phage infection compared to late strains (AA43, AA44). In contrast, the susceptibility to phages did not change during 96-month colonization by AMT 0023 strains; these strains were infected by LPS dependent phages KT28, KTN6 and additionally by LBL3 phage. The phage patterns were almost identical for the mucoid and non-mucoid pair IST 27 and IST 27N.

**Antibiotic resistance**

There was considerable variability in antibiotic susceptibilities within the panel (Table S4). As expected, all strains were sensitive to colistin. With the exception of ticarcillin-clavulanic acid and ofloxacin, proportionately more strains were susceptible than were resistant to the antibiotics tested. In general, CF strains showed resistance to more antibiotics tested than non-CF strains, as expected. Only five CF strains were susceptible to most, but not all antibiotics. Among these susceptible strains, resistance was exhibited as follows: DK2 was resistant to ticarcillin-clavulanic acid; AMT0023-30 and U018a were intermediate to aztreonam; RP1 resistant to piperacillin-tazobactam and ticarcillin-clavulanic acid, while intermediate to aztreonam; the susceptibility of the non-mucoid strain ISTN towards aztreonam was considered indeterminate. Its mucoid variant was

susceptible to all antibiotics tested. Not surprisingly, four of these susceptible CF strains were early paediatric strains.

**Presence of the *ureC* gene and quantification of urease activity**

Many bacteria utilise urease to survive in acidic conditions or as a nitrogen source. It is essential to colonisation of many bacterial pathogens, including *Helicobacter pylori* and *Pseudomonas* spp. (Konieczna *et al.*, 2012). All strains produced urease, but it was variable and depended on culture conditions (Fig. S4).

## DISCUSSION

The international panel was assembled to reflect the diversity of sources and geographical origins across *P. aeruginosa*, providing a useful resource for researchers investigating *P. aeruginosa* pathogenesis or novel therapies against this organism (De Soyza *et al.*, 2013). The variability in phenotypes demonstrated in this study, highlights the diversity of the panel strains and *P. aeruginosa* itself. Considerable genome diversity was previously documented in a series of chronic CF strains (Mowat *et al.*, 2011). In this study, both CF and non-CF groups within the panel showed considerable phenotypic variability across the parameters measured.

The only statistically significant difference between the CF and non-CF strains was the lower virulence of CF strains in the *G. mellonella* model. This would not be unexpected as *G. mellonella* is an acute infection model and CF strains tend to accumulate mutations in virulence factors which may be important for acute infections (Cullen & McClean, 2015; Sousa & Pereira, 2014). It has been demonstrated by others (Bragonzi *et al.*, 2009; Lore *et al.*, 2012) and also in this study that *P. aeruginosa* strains show reduced virulence over time of colonisation, most likely to avoid detection in the host. Since CF strains generally were less virulent, the reduced virulence in *G. mellonella* may reflect an early adaptation during colonisation that would enable long-term colonisation and chronic infection. Overall, the previously reported mucoid strains were among the least virulent strains examined with 96833S, 2192 and NH573888A each showing LD50 values between 500 and 250,000 CFU. LPS expression patterns also dramatically correlated with virulence for the majority of low virulence strains. Most strains showing low levels of virulence (LD50s greater than 650 CFU) produced no or very little O-antigen. However, there are other factors at play in the virulence mechanisms, as DK2 also showed no O-antigen expression, yet was considerably virulent in *G. mellonella*. Virulence was independent of the serotypes represented in the panel with comparable very low LD50 values being observed for serotypes 1, 11, 12 and 17.

The motility of the strains in the panel was highly variable. The major difference was associated with the CF strains, which demonstrated a lack of motility characteristic of adaptation to chronic lung infection (Mahenthiralingam *et al.*, 1994). In addition, although swarming motility has been characterised for various *P. aeruginosa* strains (Overhage *et al.*, 2008; Rashid & Kornberg, 2000) and UCBPP-PA14 (Tremblay & Deziel, 2010), only two panel strains demonstrated a true surface swarming phenotype. Swarming was highly dependent on growth media and conditions; hence the variation in phenotype observed compared to published literature may have been due to local test conditions. The growth of the reference strains was also quite variable. The transmissible strains LES B58, LES 400, LES 431 and C3719, showed unusual growth curves and interestingly, these four transmissible strains are also non-motile. Another transmissible strain, AUS 52, was among the

group of strains with low culture density and was also non-motile. In contrast, transmissible strain DK2, was considerably motile and was grouped among the strains with high culture density. The sequential strains AMT 0060-1, 2 and 3 and the AA2, AA43 and AA44 series each retained the relatively high culture density despite time of colonisation, indicating that this attribute is not altered over time of colonisation.

Previous studies on biofilm formation were performed on a small number of the panel strains, but varied in the experimental parameters used, including culture media, time intervals tested and substrata used (Carter *et al.*, 2010; Colvin *et al.*, 2011; Junker & Clardy, 2007; Kukavica-Ibrulj *et al.*, 2008; Mikkelsen *et al.*, 2013; Mulcahy *et al.*, 2010; Zegans *et al.*, 2012; Zhang *et al.*, 2013). Our comprehensive panel strain analysis carried out over 3 days and using 3 different media indicated that all strains in the panel could form biofilms. Swarming motility is inversely related to biofilm forming potential (Verstraeten *et al.*, 2008); however no such trend was observed in this study since certain non-swarming strains were poor biofilm formers while others formed biofilms very well (Table 1 and Table 3S).

Pyocyanin production is QS-controlled. Fluctuations in pyocyanin production in a series of 40 LES strains were reported, with overproduction during exacerbations in some CF patient strains and loss of pyocyanin during exacerbations in others (Fothergill *et al.*, 2010). Down-regulation of pyocyanin production was associated with a mucoid to non-mucoid switch (Ryall *et al.*, 2014). Consistent with this, the mucoid strain IST 27 produced less pyocyanin relative to the spontaneous non-mucoid variant, IST 27N. The most virulent strains produced the highest amount of pyocyanin and showed LD50 values in *G. mellonella* of less than 1 CFU at 24 h. The relatively low levels of pyocyanin in the LES strains and other CF strains, AUS 52, AMT-0060 and bronchiectasis strain 968333S correlates to the relative low virulence of these strains in *G. mellonella*. A strong correlation between pyocyanin production and virulence was confirmed for the entire panel (Spearman rank correlation coefficient $R = 0.36$, $p < 0.02$). In contrast to earlier studies (Hendrickson *et al.*, 2001; Sonnleitner *et al.*, 2003), these data indicate that pyocyanin may contribute to virulence in the *G. mellonella*, as shown recently (Whiley *et al.*, 2014).

The mucoid phenotype is often reported as being unstable and non-mucoid variants can emerge both during culture and in the CF lung through suppressor mutations. While investigating the mucoid strain IST27, care had to be taken to ensure that the mucoid phenotype was maintained. Culture in LB prior to virulence assays resulted in loss of the mucoid phenotype with a consequent enhancement of virulence. In order to maintain the mucoid phenotype, it is important to cultivate these strains in PIA as the difference between mucoid and non-mucoid phenotypes is not as distinct

when strains are grown on LB agar. Care must be taken to select colonies with well-defined mucoid or non-mucoid phenotype (depending on the strain) to perform phenotypic characterisations.

Twitching motility is driven by extension, tethering and retraction of Type IV pili (Mattick, 2002). One of the late AMT-0060 strains (AMT 0060-2) showed both a lack of twitching motility and a resistance to the type IV-dependent phage Luz19, in contrast to both AMT0060-1 and AMT0060-3, confirming that type IV have been lost in this series. Interestingly, both CF late strains (AA43 and AA44) did not differ in phage typing patterns from each other, which was confirmed by motility and LPS characterization. Moreover, the phenotypic modification during persistence of infection did not affect the activity of LBL3 phage in this series of paediatric strains. The presence of twitching motility and identical LPS bands patterns confirmed the phage specificity to recognize type IV pili and LPS elements. However, three of the 16 non-twitching strains (968333S, NH57388A and LMG14084) were susceptible to respectively four, three and two of IV-pili dependent phages (LUZ 19, ϕKZ, KTN4 and PA5oct), indicating that they retained type IV receptors, though lacking the ability to twitch. This could imply that although the type IV pili are present and act as phage receptors, they may have lost the motility function. Alternatively, utilization of other receptors by phages cannot be ruled out.

There are many strategies of bacterial resistance to phages, which may explain these results. Bacteriophages are highly specific, usually infecting strains within a single bacterial species. The specificity of interactions between phage and host cell surface receptors greatly influences the bacterial host range (Sulakvelidze *et al.*, 2001; Weinbauer, 2004). *P. aeruginosa* receptors include LPS, outer membrane proteins, oligosaccharides, capsule, type IV fimbriae, flagella and sex pilus (Guttman, 2005). It should be stressed that the most common mechanism of bacterial resistance to phage infection involves the lack, modification or masking of a target receptor, which blocks phage adsorption on the bacterial surface and results in complete loss of the ability to generate virus progeny. Moreover, bacteria can inhibit the phage cycle at other crucial steps of the propagation process, as recently reviewed (Drulis-Kawa *et al.*, 2012; Labrie *et al.*, 2010). Overall, the phage typing patterns were consistent with twitching motility and LPS analysis. The selected phages were active against most of CF and non-CF panel strains and the typing patterns correlated with bacterial cell surface elements presence such as IV-type pili and LPS structure.

The considerable antibiotic resistance across the panel was expected, with CF strains generally showing resistance to more antibiotics than non-CF strains. The only antibiotic that all strains were susceptible to was colistin, which remains a last-resort antibiotic for *P. aeruginosa* treatment. Sensitivity to colistin is a hallmark of *P. aeruginosa* and consequently this antibiotic has

been used to distinguish *P. aeruginosa* strains from another CF associated pathogen, *Burkholderia cepacia* complex, which can grow in the presence of the colistin.

In summary, this panel demonstrates the remarkable diversity seen across *P. aeruginosa* as a species. The panel includes several transmissible strains, which generally show very low pyocyanin levels, low virulence and a lack of O-antigen or B-bands. Furthermore it contains three sets of sequential strains which also show reduced virulence over time of colonisation, reduced pyocyanin and reduced O-antigen expression. Finally, the population of CF strains in the *P. aeruginosa* reference panel shows lower virulence compared with the remaining strains in the panel.

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737 **Table 1. Summary table of phenotypes including growth density, virulence, motility, pyocyanin production and alginate production.**

| Strain designation | LMG number | Short Description* | Growth (Density at 15 h) | Virulence† | Swimming§ diameter (mm) | Swarming diameter (mm) § | | Twitching diameter (mm) § | Pyocyanin‡ | Alginate +/- |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | LB 0.5% agar | BSM-G 0.5% agar | | | |
| LES B58 | 27622 | CF, transmissible | L | L | 17.5 | - | - | - | H | - |
| LES 400 | 27623 | | L | L | - | - | - | - | VL | - |
| LES 431 | 27624 | | L | L | - | - | - | - | VL | - |
| C3719 | 27625 | | L | L | - | - | - | - | H | - |
| DK2 | 27626 | CF, transmissible, early isolate | H | L | 53.5 | 19 | 12.5 | 12 | L | - |
| AES-1R | 27627 | CF transmissible paediatric | L | H | 51 | - | - | - | VL | - |
| AUS23 | 27628 | CF transmissible adult | L | L | 52.5 | - | - | 9 | | - |
| AUS52 | 27629 | | L | H | - | - | - | - | | - |
| AA2 | 27630 | Early CF | H | H | 67 | 11 | 10 | 24.5 | H | - |
| AA43 | 27631 | Late CF | H | H | 67 | 15.5 | 9 | 25 | VL | - |
| AA44 | 27632 | Late CF | H | H | 60 | 14.5 | 7.5 | 29.5 | VL | - |
| AMT 0023-30 | 27633 | Paediatric CF early | H | H | 53.5 | 12 | 9 | 27.5 | H | - |
| AMT 0023-34 | 27634 | Paediatric CF late | I | L | 53.5 | - | - | - | VL | - |
| AMT 0060-1 | 27635 | Paediatric CF late | H | L | 46.5 | 15.5 | 8.5 | 12.5 | L | - |
| AMT 0060-2 | 27636 | Paediatric CF late | H | H | 31.5 | - | - | - | L | + |
| AMT 0060-3 | 27637 | Paediatric CF early | H | L | 61 | 24.5 | 14 | 12.5 | H | - |
| PAO1* (ATCC 15692) | 27638 | Non CF | H | H | 60.5 | 28 | 18.5 | 23.5 | H | - |
| UCBPP-PA14 | 27639 | Non CF | L | H | 62.5 | 24 | 16.5 | 11 | VL | - |
| PAK | 27640 | Non CF | H | H | 60 | 12.5 | 10 | 17 | VL | - |
| CHA | 27641 | CF | H | H | 80 | 25.5 | 18 | 19.5 | L | + |
| IST 27 mucoid | 27643 | CF | H | M | 51.5 | 13.5 | Swarming | 5.5 | L | + |
| IST 27 non-mucoid | 27644 | CF | H | H | 72 | 21.5 | Swarming | 22.5 | L | - |
| 968333S | 27645 | Non-CF Bronchiectasis | L | L | - | - | - | - | VL | + |
| 679 | 27646 | Non-CF urine | H | H | 57 | 13.5 | 17.5 | - | H | - |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 39016 | 27647 | Non-CF eye | H | H | 75 | 22.5 | 11 | 30.5 | H | |
| 2192 | 27648 | CF | H | L | Spread all over plate | 11 | 21.5 | - | H | + |
| NH57388A | 27649 | CF | L | L | 7.5 | 9 | 8.5 | - | VL | - |
| 1709-12 | 27650 | Non CF clinical | H | H | 29.5 | 7.5 | - | - | H | - |
| Mil 162 | 27651 | Non-CF burn | L | H | 62.5 | - | - | - | VL | - |
| Jpn 1563 | 27652 | Water | H | H | 25 | 16.5 | 15 | 38 | L | - |
| LMG 14084 | 27653 | Water | H | H | 58.5 | 14.5 | - | - | H | - |
| Pr335 | 27654 | Hospital environment | H | H | 53.5 | 16 | 11.5 | 18.5 | H | - |
| U018a | 27655 | CF | H | H | 53.5 | 28.5 | 16.5 | 22.5 | H | - |
| CPHL 9433 | 27656 | Tobacco plant | H | H | 66 | 13.5 | 7.5 | 11.5 | H | - |
| RP1 | 27657 | CF | H | H | 65 | 26 | 8.5 | 15.5 | VL | - |
| 15108/1 | 27658 | Non-CF ICU | H | H | 61 | 34 | 17.5 | 14.5 | VL | - |
| 57P31PA | 27659 | Non-CF, COPD | H | H | 68.5 | 31 | 14 | 14 | L | - |
| 13121/1 | 27660 | Non-CF ICU | H | H | 54.5 | 16 | 9.5 | 6.5 | VL | - |
| 39177 | 27661 | Non-CF eye | H | H | 48.5 | 15.5 | 8 | 19.5 | H | - |
| KK1 | 27662 | CF | H | H | 74.5 | 17.5 | 11 | 21 | L | - |
| A5803 | 27663 | Community acquired pneumonia | H | H | 55.5 | 14 | 10.5 | 14 | H | - |
| TBCF 10839 | 27664 | CF | H | H | 67.5 | 36.5 | 32.5 | - | H | - |

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739 * Full description in (De Soyza *et al.*, 2013). † Virulence summarised as "H" representing high virulence, LD50 <5 CFU; "M" representing medium virulence,
740 i.e LD50 >5<650 CFU and "L">representing low virulence, LD50>650 CFU. ‡ Pyocyanin summarised as high > 0.1, low <0. 1 and very low <0.05; § Shading
741 designates "highly motile".

742 Table 2: Phage typing of *P. aeruginosa* panel.*

| Strain designation | Source | Phages | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | LUZ 7 | LUZ 19 | LBL 3 | KT28 | KTN6 | φKZ | KTN4 | PA5oct |
| LES 400 | CF | | | + | | | | | |
| LES 431 | CF | | | + | | | | | |
| LES B58 | CF | | | + | | | | | |
| C3719 | CF | +/- | | +/- | +/- | +/- | | | |
| DK2 | CF | | +/- | | | | +/- | +/- | |
| AES-1R | CF | +/- | +/- | | | | | | |
| AUS 23 | CF | | +/- | | | | + | | |
| AUS 52 | CF | +/- | | +/- | +/- | +/- | +/- | | |
| AA2 | CF | +/- | | | | | | | |
| AA43 | CF | +/- | | | +/- | +/- | +/- | + | +/- |
| AA44 | CF | +/- | | | +/- | +/- | +/- | | +/- |
| AMT 0023-30 | CF | + | | +/- | +/- | +/- | | + | |
| AMT 0023-34 | CF | | | +/- | +/- | +/- | | | |
| AMT 0060-1 | CF | | +/- | +/- | | | | | |
| AMT 0060-2 | CF | | | +/- | | | | | |
| AMT 0060-3 | CF | | +/- | +/- | | | | +/- | |
| PAO1* | CLIN | +/- | +/- | | +/- | +/- | +/- | +/- | +/- |
| UCBPP-PA14 | CLIN | | | + | | | +/- | | |
| PAK | CLIN | +/- | +/- | +/- | +/- | +/- | +/- | +/- | |
| CHA | CF | +/- | +/- | +/- | +/- | +/- | | +/- | + |
| IST 27 mucoid | CF | +/- | +/- | | | +/- | +/- | +/- | |
| IST 27N | CF | +/- | +/- | | + | +/- | +/- | +/- | |
| 968333S | CLIN | +/- | +/- | | | | +/- | +/- | + |
| 679 | CLIN | +/- | | | +/- | +/- | | | |
| 39016 | CLIN | | | | | | | | |
| 2192 | CF | | | | | | | | |
| NH57388A | CF | | +/- | +/- | | | +/- | | + |
| 1709-12 | CLIN | | | | | | | | |
| Mi162 | CLIN | +/- | | | | | | | |
| Jpn 1563 | ENV | +/- | +/- | | | | +/- | +/- | +/- |
| LMG 14084 | ENV | | +/- | | | | + | | |
| Prr 335 | ENV | | +/- | | | | +/- | +/- | |
| U018a | CF | | +/- | | +/- | + | | | + |
| CPHL 9433 | ENV | | | +/- | | +/- | + | | |
| RP1 | CF | | | | | | | | |
| 15108/1 | CLIN | | +/- | | | | + | +/- | |
| 57P31PA | CLIN | | +/- | | | +/- | | | |
| 13121/1 | CLIN | | | | | | +/- | | |
| 39177 | CLIN | | | | | | | | |
| KK1 | CF | + | | +/- | | +/- | | | + |
| A5803 | CLIN | +/- | +/- | +/- | | +/- | + | + | + |
| TBCF10839 | CF | | | | | +/- | + | | |

743 *Symbols: + indicates confluent clear lysis; +/- indicates confluent opaque lysis; empty boxes represent lack of
744 activity.
745

## Figure Legends:

**Fig. 1. Growth of *P. aeruginosa* panel strains.** a) Maximum culture density reached at 15 h growth. The distribution of the maximum culture density data at 15 hours growth were visualised using a boxplot displaying median, upper quartile, lower quartile, maximum and minimum values. Data points for each of the strains are included on the plot. The black brackets illustrate three broad strain groupings (high, intermediate and low culture density) and the strain names are listed in order (highest to lowest Max OD) to the right of the boxplot. b) Growth parameters at 30 h growth. The distribution of the growth parameter data (length of lag phase, growth rate and maximum culture density reached) were visualised using boxplots which display the median, upper quartile, lower quartile, maximum and minimum values. Outliers are indicated by open circles. The 36 strains included in the analysis have either been grouped together (All) or by isolation source (CF; CLIN; ENV). Strain names have been included next to outliers. Each experiment was performed twice with four technical replicates per strain.

**Fig 2. Virulence in *G. mellonella.*** Virulence was measured in terms of % survival of groups of 6 larvae in at least two independent experiments. The mean LD50 (the CFU that resulted in 50% killing of the larvae) at 24 hours is presented +/- standard deviation.

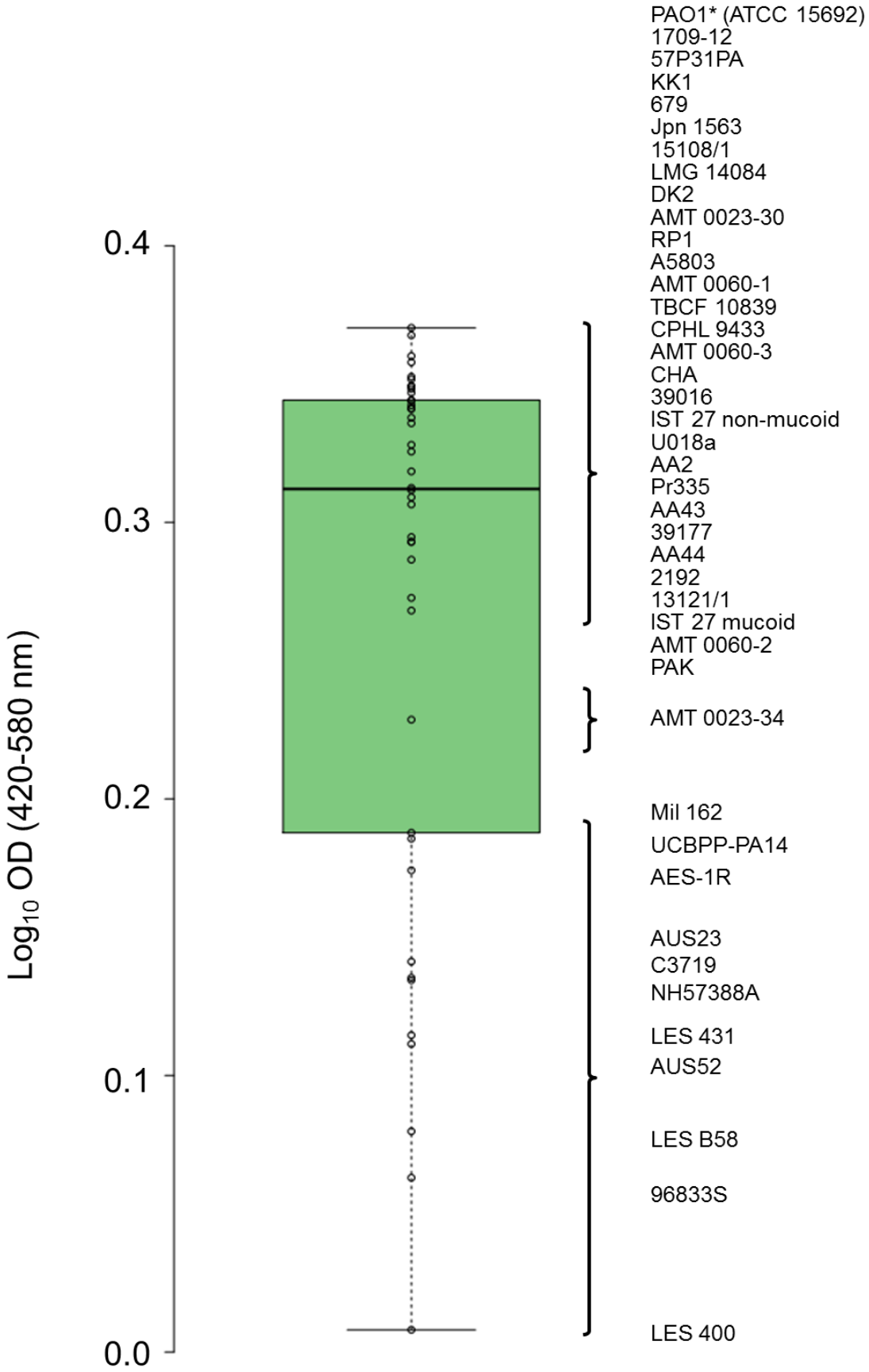
**Fig. 3. AHL expression.** Signal obtained using the *P. aeruginosa* QSIS2 biosensor (a) or the *E. coli* JB523 biosensor (b) with supernatants from 24 h *P. aeruginosa* cultures. The results are presented as mean +/- SEM of four independent replicates. $^{*}$Significantly different from negative control ($p < 0.05$).

**Fig. 4. Biofilm formation as determined by crystal violet staining.** Results are presented as mean maximal absorbance at A570nm for each strain (+/- standard deviation) from at least two independent experiments. The medium that resulted in maximal biofilm formation together with the timepoint at which this was registered is identified in parenthesis, where 1= 24 h, 2= 48 h and 3= 72 h).

**Fig. 5. Pyocyanin production.** Pyocyanin was extracted in chloroform, back-extracted in HCl and measured at 520nm. The results are the means of at least two independent experiments performed in duplicate ± SEM.

**Fig. 6**. **LPS profiles following SDS-PAGE**. LPS was extracted from overnight cultures at $OD_{600}$ of 2.0 and separated on 14% polyacrylamide/Glycine-SDS gels. The majority of strains express smooth forms of LPS and display a ladder profiles that are strain-specific.

778 **Figure 1 (a)**

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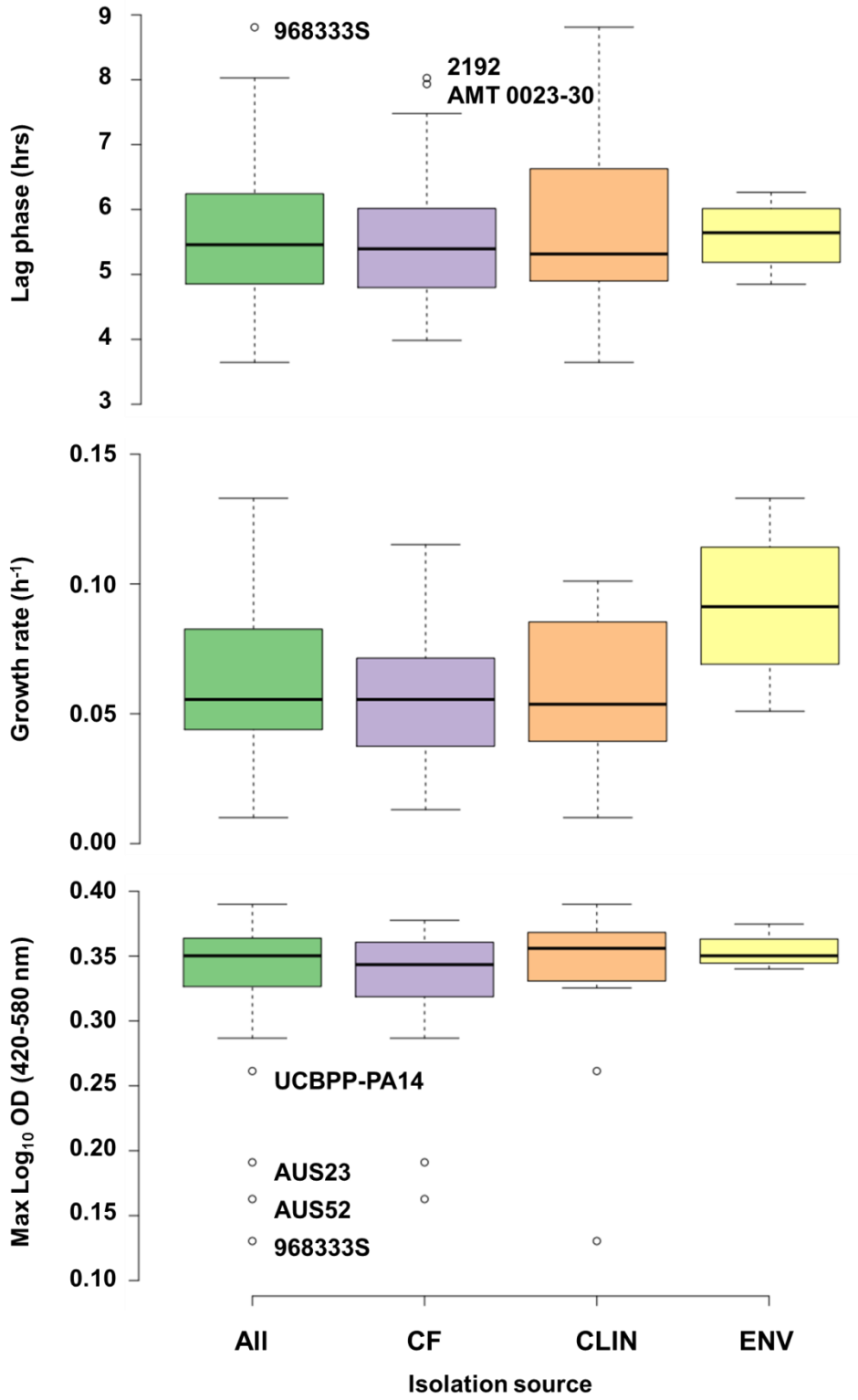
780 **Fig 1(b)**

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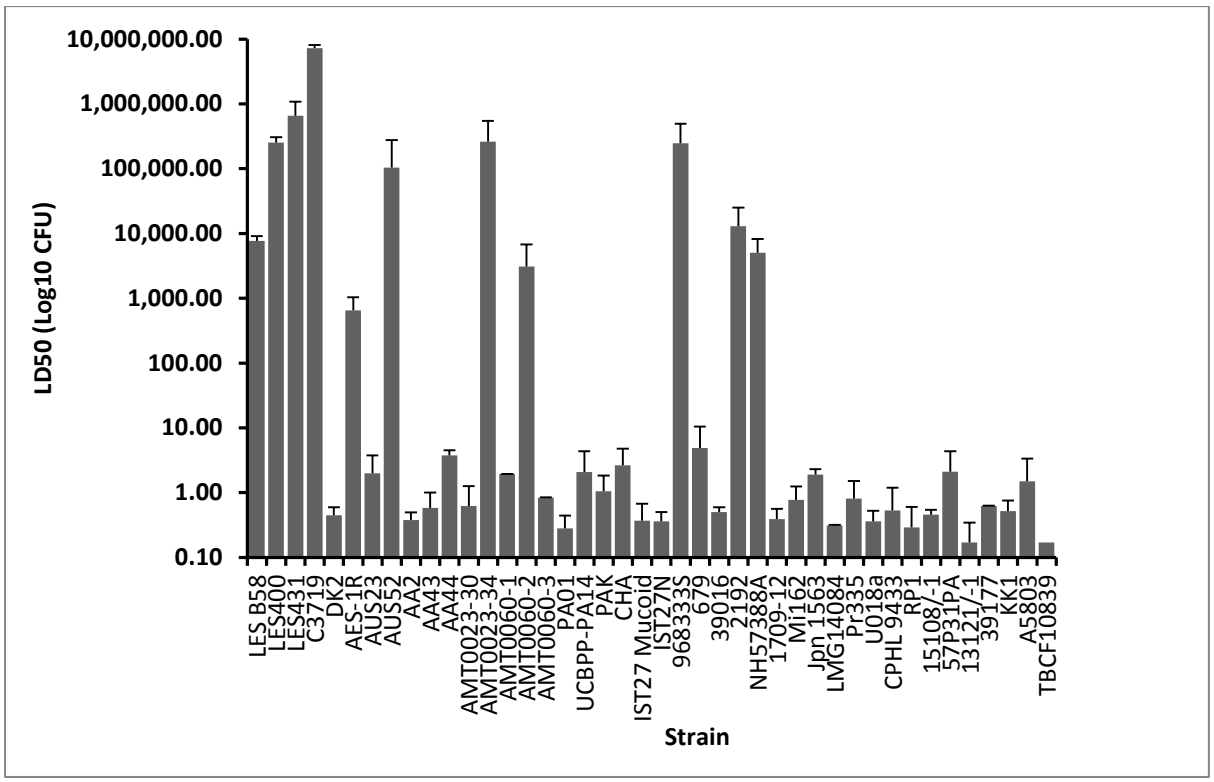
785 **Figure 2**

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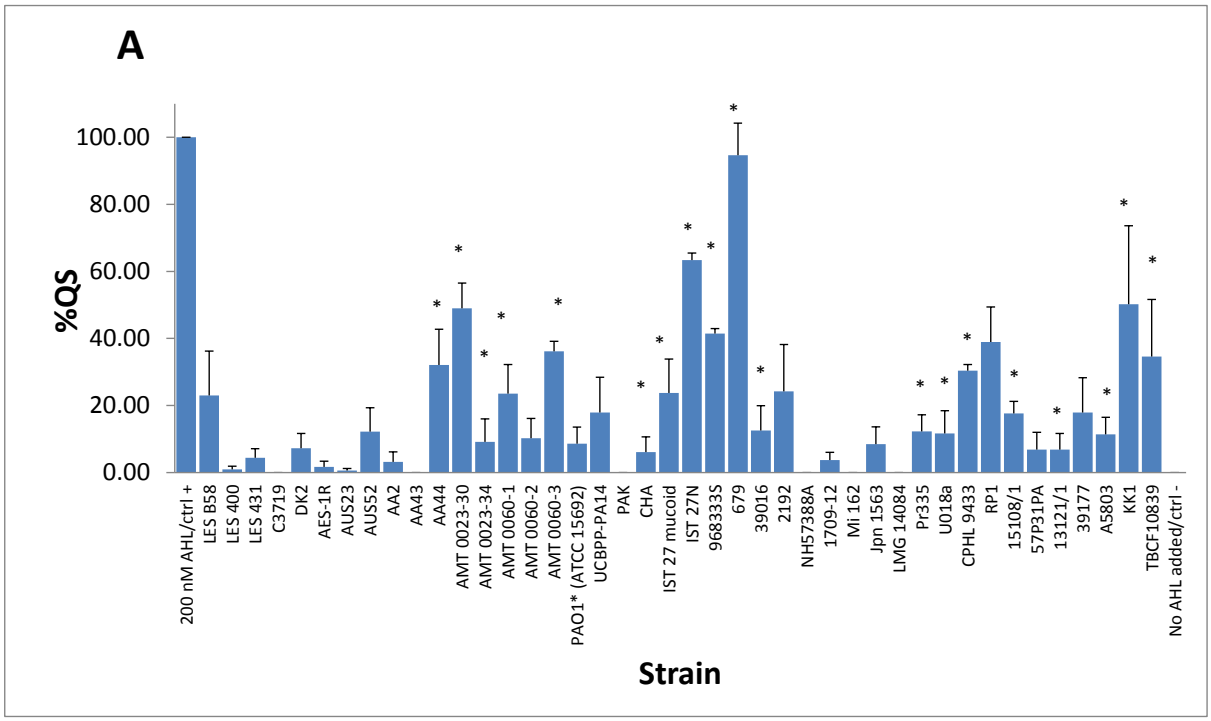
787

## Figure 3:


A
%QS
100.00
80.00
60.00
40.00
20.00
0.00
200 nM AHL/ctrl +
LES B58
LES 400
LES 431
C3719
DK2
AES-1R
AUS23
AUS52
AA2
AA43
AA44
AMT 0023-30
AMT 0023-34
AMT 0060-1
AMT 0060-2
AMT 0060-3
PAO1* (ATCC 15692)
UCBPP-PA14
PAK
CHA
IST 27 mucoid
IST 27N
968333S
679
39016
2192
NH57388A
1709-12
Mi 162
Jpn 1563
LMG 14084
Pr335
U018a
CPHL 9433
RP1
15108/1
57P31PA
13121/1
39177
A5803
KK1
TBCF10839
No AHL added/ctrl -
Strain

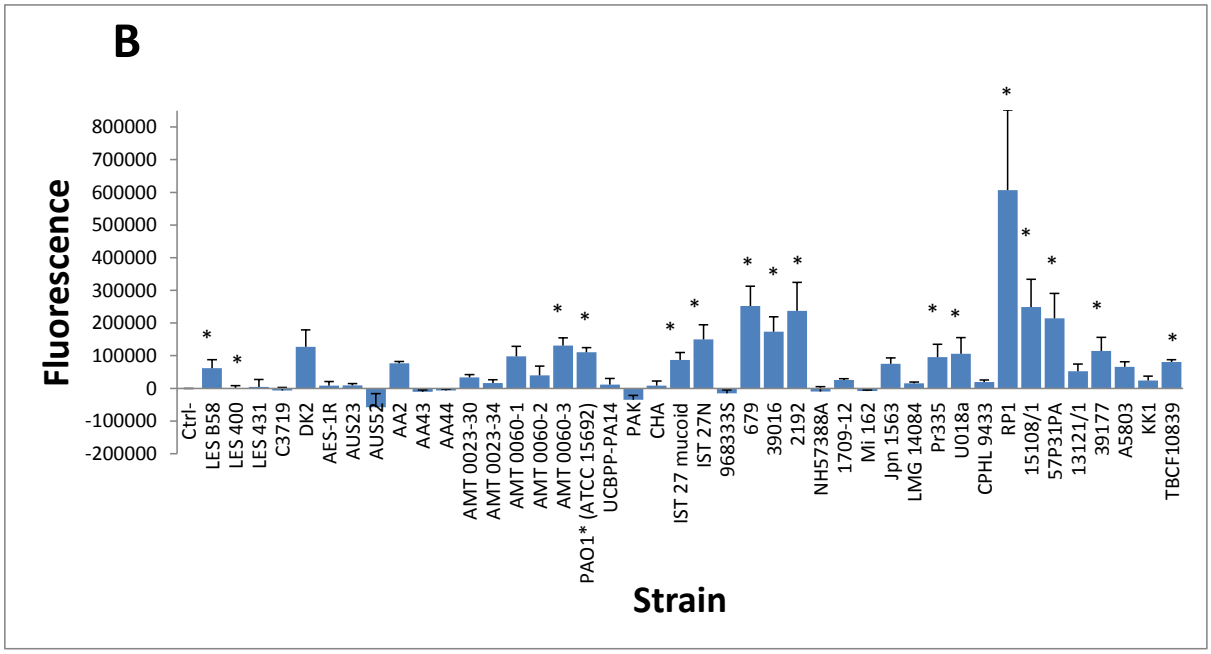
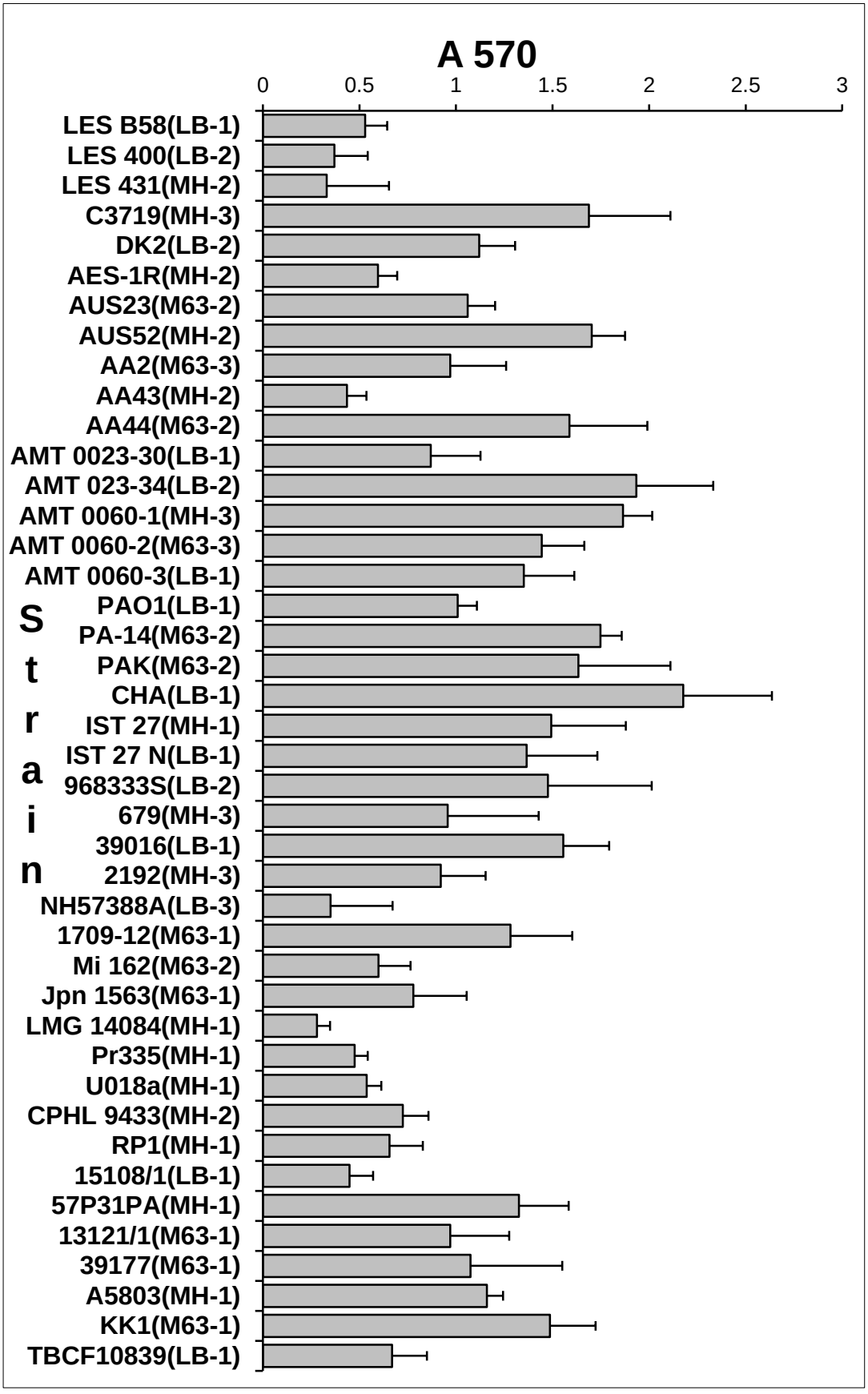
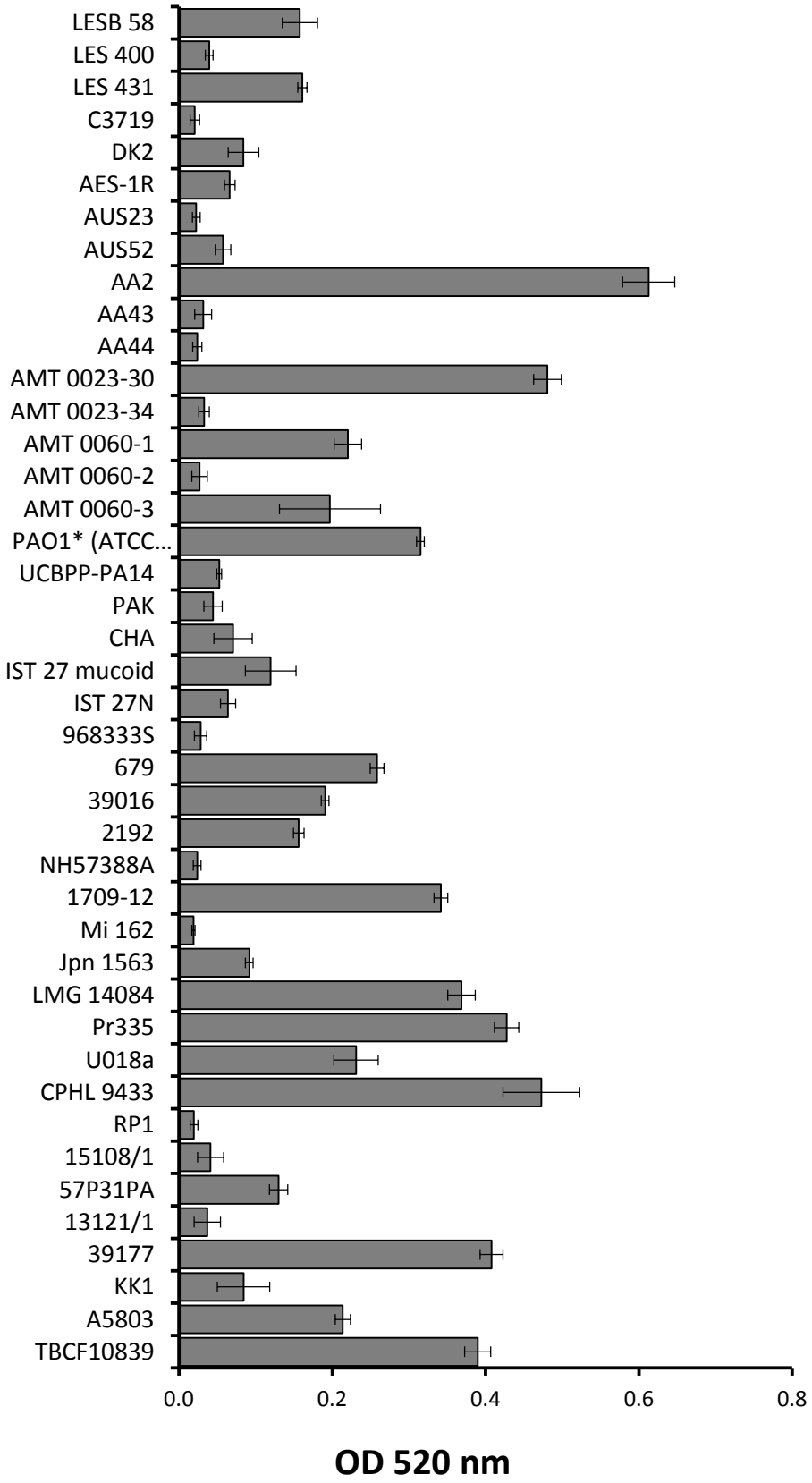

B
Fluorescence
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
-200000
Ctrl-
LES B58
LES 400
LES 431
C3719
DK2
AES-1R
AUS23
AUS52
AA2
AA43
AA44
AMT 0023-30
AMT 0023-34
AMT 0060-1
AMT 0060-2
AMT 0060-3
PAO1* (ATCC 15692)
UCBPP-PA14
PAK
CHA
IST 27 mucoid
IST 27N
968333S
679
39016
2192
NH57388A
1709-12
Mi 162
Jpn 1563
LMG 14084
Pr335
U018a
CPHL 9433
RP1
15108/1
57P31PA
13121/1
39177
A5803
KK1
TBCF10839
Strain

**Figure 4**

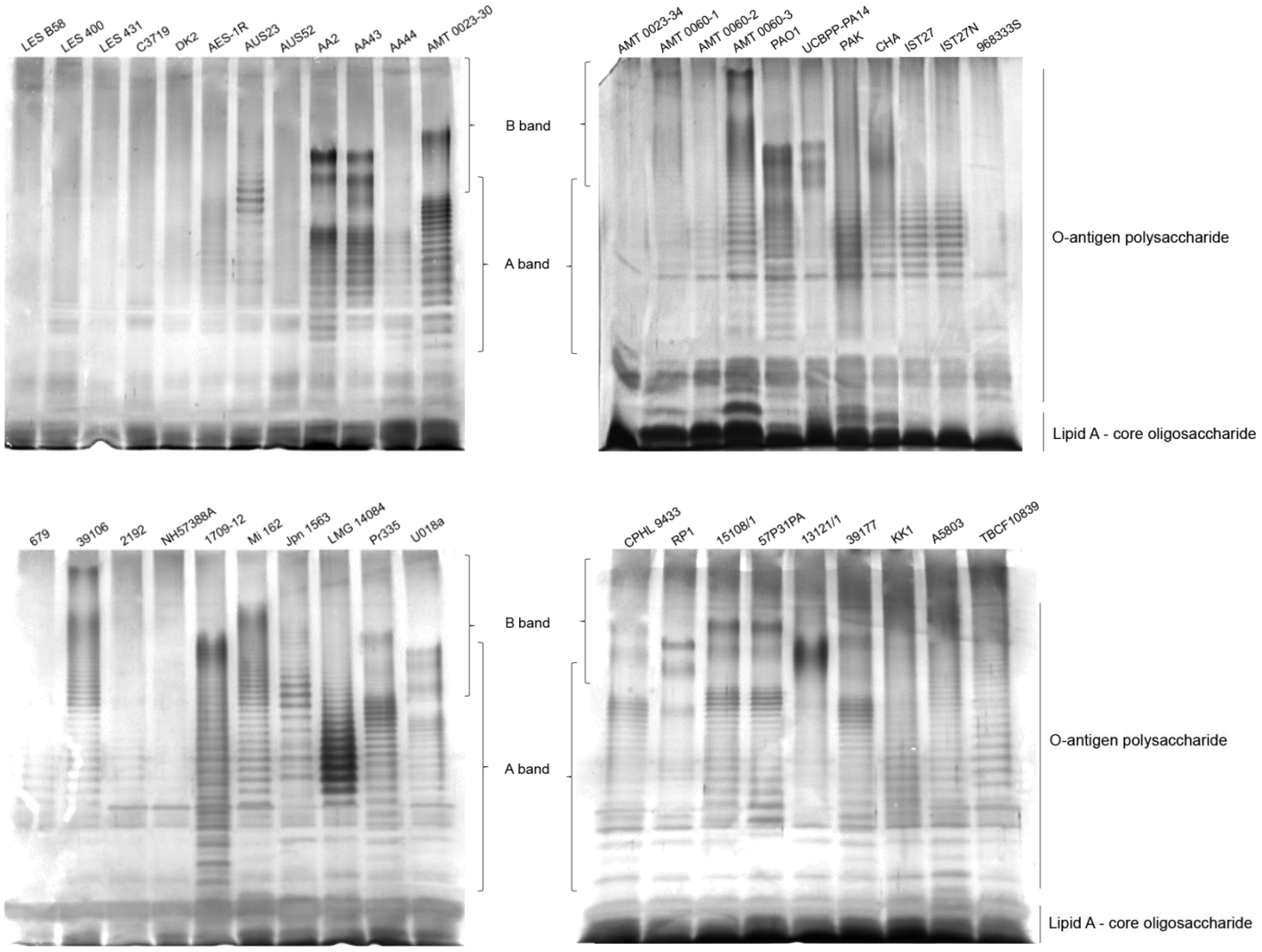

A 570
0
0.5
1
1.5
2
2.5
3
LES B58(LB-1)
LES 400(LB-2)
LES 431(MH-2)
C3719(MH-3)
DK2(LB-2)
AES-1R(MH-2)
AUS23(M63-2)
AUS52(MH-2)
AA2(M63-3)
AA43(MH-2)
AA44(M63-2)
AMT 0023-30(LB-1)
AMT 023-34(LB-2)
AMT 0060-1(MH-3)
AMT 0060-2(M63-3)
AMT 0060-3(LB-1)
PAO1(LB-1)
PA-14(M63-2)
PAK(M63-2)
CHA(LB-1)
IST 27(MH-1)
IST 27 N(LB-1)
968333S(LB-2)
679(MH-3)
39016(LB-1)
2192(MH-3)
NH57388A(LB-3)
1709-12(M63-1)
Mi 162(M63-2)
Jpn 1563(M63-1)
LMG 14084(MH-1)
Pr335(MH-1)
U018a(MH-1)
CPHL 9433(MH-2)
RP1(MH-1)
15108/1(LB-1)
57P31PA(MH-1)
13121/1(M63-1)
39177(M63-1)
A5803(MH-1)
KK1(M63-1)
TBCF10839(LB-1)
Strain




798 **Figure 5**

799

**Figure 6**


LES B58
LES 400
LES 431
C3719
DK2
AES-1R
AUS23
AUS52
AA2
AA43
AA44
AMT 0023-30
B band
A band
AMT 0023-34
AMT 0060-1
AMT 0060-2
AMT 0060-3
PAO1
UCBPP-PA14
PAK
CHA
IST27
IST27N
968333S
O-antigen polysaccharide
Lipid A - core oligosaccharide
679
39106
2192
NH57388A
1709-12
MI 162
Jpn 1563
LMG 14084
Pr335
U018a
B band
A band
CPHL 9433
RP1
15108/1
57P31PA
13121/1
39177
KK1
A5803
TBCF10839
O-antigen polysaccharide
Lipid A - core oligosaccharide

**Supplemental information.**

Table 1S: Phage used for phage typing

| Bacteriophage | Family | Description | Genome size (kbp) | GenBank Accession number |
|---|---|---|---|---|
| LUZ7 | *Podoviridae* | *N4likevirus* | 74.9 | NC_013691.1 |
| LUZ19 | *Podoviridae* | *Phikmvlikevirus* | 43.5 | NC_010326.1 |
| LBL3 | *Myoviridae* | *Pbunalikevirus* | 64.4 | NC_011165.1 |
| ϕKZ, , | *Myoviridae* | *Phikzlikevirus* | 230.3 | AF399011.1 |
| ϕKT28, | *Myoviridae* | *Pbunalikevirus* | 66.4 | KP340287 |
| ϕKTN6, | *Myoviridae* | *Pbunalikevirus,* | 66.4 | KP340288 |
| ϕKTN4, | *Myoviridae* | *Phikzlikevirus,* | 279.7 | pending |
| PA5oct, , | *Myoviridae* | NovelGiant | 287.2 | Pending |

**Table 2S. Growth parameters of 36 *Pseudomonas aeruginosa* international reference panel strains at 30 hours growth**

| Panel # | Source | Strain | Growth rate ($h^{-1}$) | Lag phase (hrs) | Max $Log_{10}$ OD (420-580 nm) |
|---|---|---|---|---|---|
| 5 | CF | DK2 | 0.07 | 4.96 | 0.36 |
| 6 | CF | AES-1R | 0.02 | 6.76 | 0.30 |
| 7 | CF | AUS23 | 0.02 | 3.98 | 0.19 |
| 8 | CF | AUS52 | 0.01 | 5.50 | 0.16 |
| 9 | CF | AA2 | 0.12 | 5.22 | 0.38 |
| 10 | CF | AA43 | 0.10 | 5.81 | 0.34 |
| 11 | CF | AA44 | 0.05 | 4.65 | 0.31 |
| 12 | CF | AMT 0023-30 | 0.10 | 4.73 | 0.35 |
| 13 | CF | AMT 0023-34 | 0.03 | 7.93 | 0.29 |
| 14 | CF | AMT 0060-1 | 0.06 | 5.37 | 0.34 |
| 15 | CF | AMT 0060-2 | 0.05 | 6.22 | 0.36 |
| 16 | CF | AMT 0060-3 | 0.06 | 4.55 | 0.36 |
| 17 | CLIN | PAO1* (ATCC 15692) | 0.07 | 5.14 | 0.37 |
| 18 | CLIN | UCBPP-PA14 | 0.02 | 3.64 | 0.26 |
| 19 | CLIN | PAK | 0.03 | 3.68 | 0.36 |
| 20 | CF | CHA | 0.04 | 4.86 | 0.34 |
| 22 | CF | IST 27 mucoid | 0.04 | 5.77 | 0.33 |
| 23 | CF | IST 27 non-mucoid | 0.05 | 5.80 | 0.33 |
| 24 | CLIN | 96833S | 0.01 | 8.81 | 0.13 |
| 25 | CLIN | 679 | 0.10 | 6.74 | 0.35 |
| 26 | CLIN | 39016 | 0.05 | 6.52 | 0.37 |
| 27 | CF | 2192 | 0.06 | 8.03 | 0.37 |
| 29 | CLIN | 1709-12 | 0.07 | 5.76 | 0.38 |
| 31 | ENV | Jpn 1563 | 0.05 | 6.26 | 0.35 |
| 32 | ENV | LMG 14084 | 0.09 | 5.76 | 0.35 |
| 33 | ENV | Pr335 | 0.10 | 4.85 | 0.34 |
| 34 | CF | U018a | 0.07 | 5.18 | 0.34 |
| 35 | ENV | CPHL 9433 | 0.13 | 5.52 | 0.37 |
| 36 | CF | RP1 | 0.08 | 4.60 | 0.37 |
| 37 | CLIN | 15108/1 | 0.05 | 6.92 | 0.33 |
| 38 | CLIN | 57P31PA | 0.05 | 4.84 | 0.39 |
| 39 | CLIN | 13121/1 | 0.05 | 5.50 | 0.34 |
| 40 | CLIN | 39177 | 0.10 | 4.98 | 0.34 |
| 41 | CF | KK1 | 0.06 | 5.42 | 0.36 |
| 42 | CLIN | A5803 | 0.10 | 4.96 | 0.36 |
| 43 | CF | TBCF 10839 | 0.07 | 7.48 | 0.37 |

Table 3S. Biofilm formation across the panel in MHB (A), LB (B) or M63 minimal medium (C) as determined by crystal violet staining at 24, 28 and 72 h. The values correspond to average absorbance (AV) and Standard deviation (STD).

**A**

| Strain | MH-24h | | MH-48h | | MH-72h | |
|---|---|---|---|---|---|---|
| | AV | STD | AV | STD | AV | STD |
| LES B58 | 0.25 | 0.04 | 0.18 | 0.04 | 0.24 | 0.06 |
| LES 400 | 0.17 | 0.06 | 0.24 | 0.07 | 0.34 | 0.17 |
| LES 431 | 0.06 | 0.02 | 0.14 | 0.06 | 0.16 | 0.06 |
| C3719 | 0.64 | 0.22 | 1.57 | 0.38 | 1.69 | 0.42 |
| DK2 | 0.29 | 0.11 | 0.34 | 0.11 | 0.23 | 0.06 |
| AES-1R | 0.09 | 0.01 | 0.60 | 0.10 | 0.19 | 0.04 |
| AUS23 | 0.30 | 0.15 | 0.46 | 0.28 | 0.16 | 0.14 |
| AUS52 | 0.48 | 0.21 | 0.56 | 0.13 | 1.70 | 0.17 |
| AA2 | 0.32 | 0.18 | 0.11 | 0.05 | 0.14 | 0.14 |
| AA43 | 0.10 | 0.07 | 0.44 | 0.10 | 0.11 | 0.06 |
| AA44 | 0.13 | 0.03 | 0.11 | 0.05 | 0.07 | 0.14 |
| AMT 0023-30 | 0.50 | 0.25 | 0.31 | 0.13 | 0.16 | 0.08 |
| AMT 023-34 | 0.29 | 0.10 | 0.12 | 0.05 | 0.44 | 0.21 |
| AMT 0060-1 | 0.66 | 0.15 | 1.62 | 0.16 | 1.87 | 0.15 |
| AMT 0060-2 | 0.17 | 0.15 | 0.09 | 0.02 | 0.17 | 0.14 |
| AMT 0060-3 | 0.43 | 0.08 | 0.00 | 0.00 | 0.26 | 0.14 |
| PAO1 | 0.31 | 0.06 | 0.19 | 0.02 | 0.23 | 0.05 |
| PA-14 | 1.46 | 0.38 | 0.74 | 0.17 | 0.43 | 0.16 |
| PAK | 0.58 | 0.22 | 0.34 | 0.12 | 0.23 | 0.13 |
| CHA | 1.10 | 0.59 | 0.54 | 0.23 | 0.28 | 0.09 |
| IST 27 mucoid | 1.49 | 0.39 | 0.48 | 0.09 | 0.17 | 0.04 |
| IST 27 N | 0.96 | 0.36 | 1.15 | 0.31 | 0.87 | 0.50 |
| 968333S | 0.10 | 0.06 | 0.14 | 0.16 | 0.11 | 0.15 |
| 679 | 0.27 | 0.04 | 0.53 | 0.16 | 0.96 | 0.47 |
| 39016 | 1.38 | 0.27 | 0.22 | 0.06 | 0.16 | 0.13 |
| 2192 | 0.50 | 0.20 | 0.71 | 0.46 | 0.92 | 0.23 |
| NH57388 A | 0.03 | 0.01 | 0.13 | 0.06 | 0.24 | 0.29 |
| 1709-12 | 0.97 | 0.34 | 0.43 | 0.09 | 0.26 | 0.07 |
| Mi 162 | 0.05 | 0.04 | 0.04 | 0.02 | 0.02 | 0.01 |
| Jpn 1563 | 0.44 | 0.18 | 0.27 | 0.04 | 0.22 | 0.02 |
| LMG 14084 | 0.28 | 0.07 | 0.11 | 0.01 | 0.04 | 0.00 |
| Pr335 | 0.48 | 0.20 | 0.32 | 0.05 | 0.23 | 0.09 |
| U018a | 0.54 | 0.07 | 0.19 | 0.02 | 0.09 | 0.02 |
| CPHL 9433 | 0.41 | 0.14 | 0.41 | 0.09 | 0.25 | 0.03 |

| | | | | | | |
|---|---|---|---|---|---|---|
| RP1 | 0.66 | 0.17 | 0.36 | 0.05 | 0.05 | 0.01 |
| 15108/1 | 0.29 | 0.07 | 0.35 | 0.16 | 0.14 | 0.05 |
| 57P31PA | 1.33 | 0.26 | 0.22 | 0.07 | 0.16 | 0.03 |
| 13121/1 | NT | NT | 0.13 | 0.04 | 0.05 | 0.02 |
| 39177 | NT | Nt | 0.15 | 0.04 | 0.09 | 0.03 |
| KK1(d1) | 1.16 | 0.24 | 0.33 | 0.08 | 0.19 | 0.04 |
| TBCF10839(d1) | 0.47 | 0.13 | 0.10 | 0.04 | 0.11 | 0.06 |

818

819 B

| | LB-24h | | LB-48 h | | LB-72 h | |
|---|---|---|---|---|---|---|
| | AV | STD | AV | STD | AV | STD |
| LES B58 | 0.90 | 0.30 | 0.21 | 0.06 | 0.11 | 0.03 |
| LES 400 | 0.28 | 0.09 | 0.37 | 0.17 | 0.17 | 0.08 |
| LES 431 | 0.05 | 0.01 | 0.07 | 0.06 | 0.07 | 0.06 |
| C3719 | 0.19 | 0.09 | 0.18 | 0.05 | 0.52 | 0.14 |
| DK2 | 1.47 | 0.33 | 0.91 | 0.21 | 0.69 | 0.12 |
| AES-1R | 0.85 | 0.28 | 0.36 | 0.04 | 0.15 | 0.04 |
| AUS23 | 0.59 | 0.12 | 0.55 | 0.16 | 0.17 | 0.06 |
| AUS52 | 0.63 | 0.23 | 0.33 | 0.15 | 0.21 | 0.06 |
| AA2 | 0.52 | 0.17 | 0.11 | 0.04 | 0.15 | 0.04 |
| AA43 | 0.07 | 0.03 | 0.19 | 0.06 | 0.06 | 0.06 |
| AA44 | 0.11 | 0.03 | 0.47 | 0.13 | 0.11 | 0.06 |
| AMT 0023-30 | 0.87 | 0.26 | 0.45 | 0.14 | 0.16 | 0.10 |
| AMT 023-34 | 1.01 | 0.30 | 1.93 | 0.40 | 1.01 | 0.52 |
| AMT 0060-1 | 0.19 | 0.04 | 0.29 | 0.06 | 0.29 | 0.07 |
| AMT 0060-2 | 0.28 | 0.05 | 0.12 | 0.03 | 0.12 | 0.02 |
| AMT 0060-3 | 1.35 | 0.26 | 0.32 | 0.11 | 0.16 | 0.08 |
| PAO1 | 1.01 | 0.10 | 0.21 | 0.06 | 0.13 | 0.07 |
| PA-14 | 1.20 | 0.35 | 1.31 | 0.33 | 0.81 | 0.35 |
| PAK | 1.48 | 0.30 | 0.80 | 0.16 | 0.84 | 0.10 |
| CHA | 2.18 | 0.46 | 1.24 | 0.44 | 0.58 | 0.13 |
| IST 27 mucoid | 1.13 | 0.49 | 0.75 | 0.28 | 0.50 | 0.23 |
| IST 27 N | 1.37 | 0.37 | 1.06 | 0.38 | 0.78 | 0.46 |
| 968333S | 1.45 | 0.54 | 1.48 | 0.37 | 0.54 | 0.45 |
| 679 | 0.82 | 0.42 | 0.37 | 0.28 | 0.23 | 0.15 |
| 39016 | 1.56 | 0.24 | 0.34 | 0.11 | 0.19 | 0.18 |
| 2192 | 0.47 | 0.19 | 0.45 | 0.11 | 0.26 | 0.16 |
| NH57388A | 1.18 | 0.30 | 0.66 | 0.11 | 0.52 | 0.11 |
| 1709-12 | 0.88 | 0.07 | 0.26 | 0.07 | 0.13 | 0.03 |
| Mi 162 | 0.15 | 0.07 | 0.22 | 0.10 | 0.11 | 0.04 |
| Jpn 1563 | 0.41 | 0.09 | 0.63 | 0.11 | 0.40 | 0.07 |
| LMG 14084 | 0.25 | 0.09 | 0.10 | 0.02 | 0.04 | 0.03 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Pr335 | 0.47 | 0.07 | 0.33 | 0.08 | 0.23 | 0.05 |
| U018a | 0.42 | 0.07 | 0.14 | 0.03 | 0.12 | 0.03 |
| CPHL 9433 | 0.22 | 0.07 | 0.72 | 0.13 | 0.66 | 0.12 |
| RP1 | 0.23 | 0.06 | 0.38 | 0.07 | 0.27 | 0.03 |
| 15108/1 | 0.45 | 0.12 | 0.31 | 0.07 | 0.14 | 0.03 |
| 57P31PA | 0.19 | 0.04 | 0.38 | 0.06 | 0.34 | 0.04 |
| 13121/1 | 0.58 | 0.04 | 0.12 | 0.01 | 0.13 | 0.07 |
| 39177 | 0.69 | 0.06 | 0.21 | 0.06 | 0.21 | 0.04 |
| KK1(d1) | 0.72 | 0.11 | 0.39 | 0.09 | 0.32 | 0.09 |
| A5803(d1) | 0.25 | 0.07 | 0.14 | 0.05 | 0.17 | 0.09 |
| TBCF10839(d1) | 0.67 | 0.08 | 0.38 | 0.09 | 0.19 | 0.06 |

820

821

822 C

| | **M63-24 h** | | **M63-48 h** | | **M63-72 h** | |
|---|---|---|---|---|---|---|
| | AV | STD | AV | STD | AV | STD |
| LES B58 | 0.07 | 0.01 | 0.05 | 0.01 | 0.10 | 0.02 |
| LES 400 | 0.22 | 0.04 | 0.19 | 0.05 | 0.13 | 0.03 |
| LES 431 | 0.05 | 0.02 | 0.07 | 0.02 | 0.15 | 0.05 |
| C3719 | 0.04 | 0.02 | 0.19 | 0.08 | 0.42 | 0.11 |
| DK2 | 1.63 | 0.25 | 0.40 | 0.14 | 0.35 | 0.12 |
| AES-1R | 0.50 | 0.10 | 0.32 | 0.06 | 0.15 | 0.08 |
| AUS23 | 0.30 | 0.09 | 1.06 | 0.14 | 0.49 | 0.09 |
| AUS52 | 1.12 | 0.43 | 0.47 | 0.17 | 0.67 | 0.16 |
| AA2 | 0.46 | 0.09 | 0.37 | 0.05 | 0.97 | 0.29 |
| AA43 | 0.18 | 0.07 | 0.43 | 0.11 | 0.08 | 0.06 |
| AA44 | 0.18 | 0.05 | 1.59 | 0.40 | 0.26 | 0.09 |
| AMT 0023-30 | 0.40 | 0.10 | 0.38 | 0.07 | 0.52 | 0.59 |
| AMT 023-34 | 1.32 | 0.28 | 1.43 | 0.80 | 1.05 | 0.40 |
| AMT 0060-1 | 0.31 | 0.09 | 0.51 | 0.10 | 0.66 | 0.10 |
| AMT 0060-2 | 0.14 | 0.13 | 0.14 | 0.17 | 0.33 | 0.05 |
| AMT 0060-3 | 0.73 | 0.40 | 0.57 | 0.19 | 0.40 | 0.15 |
| PAO1 | 0.44 | 0.10 | 0.34 | 0.04 | 0.37 | 0.10 |
| PA-14 | 3.00 | 0.08 | 2.00 | 0.30 | 1.31 | 0.26 |
| PAK | 1.18 | 0.13 | 0.92 | 0.12 | 1.31 | 0.26 |
| CHA | 0.19 | 0.04 | 0.60 | 0.09 | 0.23 | 0.08 |
| IST 27 mucoid | 0.67 | 0.15 | 0.35 | 0.14 | 0.16 | 0.08 |
| IST 27 N | 0.93 | 0.08 | 0.61 | 0.11 | 0.38 | 0.12 |
| 968333S | 0.10 | 0.07 | 1.03 | 0.17 | 0.86 | 0.16 |
| 679 | 0.53 | 0.23 | 0.72 | 0.47 | 0.53 | 0.42 |
| 39016 | 0.49 | 0.10 | 0.94 | 0.08 | 0.75 | 0.08 |
| 2192 | 0.47 | 0.07 | 0.44 | 0.16 | 0.56 | 0.17 |

| | | | | | | |
|---|---|---|---|---|---|---|
| NH57388A | 0.16 | 0.03 | 0.03 | 0.01 | 0.04 | 0.01 |
| 1709-12 | 1.28 | 0.32 | 0.22 | 0.04 | 0.11 | 0.03 |
| Mi 162 | 0.53 | 0.17 | 0.60 | 0.17 | 0.54 | 0.12 |
| Jpn 1563 | 0.78 | 0.28 | 0.44 | 0.10 | 0.17 | 0.05 |
| LMG 14084 | 0.15 | 0.03 | 0.10 | 0.02 | 0.05 | 0.01 |
| Pr335 | 0.37 | 0.13 | 0.41 | 0.14 | 0.25 | 0.10 |
| U018a | 0.31 | 0.10 | 0.27 | 0.09 | 0.24 | 0.12 |
| CPHL 9433 | 0.24 | 0.05 | 0.37 | 0.06 | 0.24 | 0.04 |
| RP1 | 0.15 | 0.03 | 0.19 | 0.05 | 0.11 | 0.02 |
| 15108/1 | 0.35 | 0.17 | 0.29 | 0.05 | 0.26 | 0.08 |
| 57P31PA | 0.15 | 0.02 | 0.19 | 0.04 | 0.18 | 0.04 |
| 13121/1 | 0.97 | 0.31 | 0.21 | 0.09 | 0.28 | 0.07 |
| 39177 | 1.07 | 0.38 | 0.21 | 0.10 | 0.17 | 0.05 |
| KK1(d1) | 0.70 | 0.14 | 0.44 | 0.07 | 0.18 | 0.02 |
| A5803(d1) | 0.95 | 0.19 | 0.60 | 0.12 | 0.57 | 0.16 |
| TBCF10839(d1) | 0.52 | 0.11 | 0.50 | 0.10 | 0.34 | 0.09 |

823
824

**Table 4S. Antibiotic resistance of the panel strains**

| | EUCAST Breakpoint | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S ≤** | 4 | 4 | 0.5 | 1 | 16 | 16 | 8 | 8 | 8 | 8 | 2 | 0.5 | 4 | 8 | 1 | 16 |
| **I** | - | - | 1 | 2 - 16 | - | - | - | - | - | - | 4 - 8 | 1 | - | 16 | 2 | - |
| **R >** | 4 | 4 | 1 | 16 | 16 | 16 | 8 | 8 | 8 | 8 | 8 | 1 | 4 | 16 | 2 | 16 |
| | **Genta micin** | **Coli stin** | **Oflox acin** | **Aztreo nam** | **Pipera cillin** | **Piperac illin-tazoba ctam** | **Cefoper azone** | **Ceftazi dime** | **Cefep ime** | **Cefop./ sulb** | **Merope nem** | **Ciproflo xacin** | **Tobra mycin** | **Amik acin** | **Levoflo xacin** | **Ticarc illin-clavul anic acid** |
| **LES B58** | R | S | R | R | S | R | S | R | R | S | Ind | R | S | R | R | R |
| **LES 400** | R | S | R | R | S | S | S | R | R | S | I | R | S | R | R | R |
| **LES 431** | R | S | R | R | R | R | R | R | R | R | I | R | S | R | R | R |
| **C3719** | S | S | R | R | R | R | R | R | R | S | R | R | S | Ind | R | R |
| **DK2** | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S | R |
| **AES-1R** | R | S | R | S | R | S | R | S | R | R | R | S | S | R | S | R |
| **AUS23** | S | S | R | I | Ind | S | R | S | S | R | S | S | S | S | S | R |
| **AUS52** | R | S | R | S | S | S | S | S | R | S | R | R | R | S | R | S |
| **AA2** | S | S | S | S | S | R | R | S | S | S | S | S | S | S | S | R |
| **AA43** | S | S | R | Ind | S | R | S | S | R | S | S | S | S | S | S | R |
| **AA44** | S | S | R | I | R | R | R | S | S | S | S | S | S | S | S | R |
| **AMT 0023-30** | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S | S |
| **AMT 0023-34** | R | S | R | S | S | S | S | S | S | S | S | Ind | R | R | R | S |
| **AMT 0060-1** | S | S | R | S | S | S | S | S | S | S | S | I | S | S | R | S |
| **AMT 0060-2** | S | S | R | I | S | S | S | S | S | S | S | S | S | S | S | S |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AMT 0060-3** | S | S | I | I | S | S | S | S | S | S | S | S | S | S | S | R |
| **PAO1*( ATCC 15692)** | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S | S |
| **UCBPP-PA14** | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S | R |
| **PAK** | S | S | S | I | S | **S** | S | S | S | S | S | S | S | S | S | R |
| **CHA** | R | S | I | I | S | S | S | S | S | S | S | S | S | S | S | R |
| **IST 27 mucoid** | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| **IST 27N** | S | S | S | Ind | S | S | S | S | S | S | S | S | S | S | S | S |
| **968333S** | R | S | I | R | R | R | R | R | R | S | I | S | S | S | S | R |
| **679** | R | S | R | I | S | S | S | S | S | S | I | S | S | S | S | R |
| **39106** | S | S | I | I | S | S | S | S | S | S | S | S | S | S | S | R |
| **2192** | S | S | S | S | Ind | S | S | S | S | S | S | S | S | S | S | S |
| **NH57388A** | S | S | R | Ind | R | S | R | R | R | S | S | R | S | S | R | R |
| **1709-12** | R | S | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| **Mi 162** | R | S | R | R | R | R | R | R | R | R | R | S | R | Ind | S | R |
| **Jpn 1563** | S | S | R | I | S | S | R | S | S | S | S | S | S | S | S | R |
| **LMG 14084** | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S | R |
| **Pr335** | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S | R |
| **U018a** | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S | R |
| **CPHL 9433** | S | S | I | I | S | S | S | S | S | S | S | S | S | S | S | S |
| **RP1** | S | S | S | I | S | R | S | S | S | S | S | S | S | S | S | R |
| **15108/1** | S | S | R | I | S | R | R | S | S | R | I | S | S | S | S | R |
| **57P31PA** | S | S | I | I | S | S | S | S | R | S | S | S | S | S | S | R |
| **13121/1** | S | S | R | I | R | R | R | 8 | S | R | S | R | S | S | R | R |
| **39177** | S | S | I | I | S | S | S | S | S | S | S | S | S | S | R | R |
| **KK1** | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S | R |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A5803** | S | S | R | I | S | R | S | S | R | S | S | R | S | S | R | R |
| **TBCF10839** | S | S | I | I | S | R | R | S | S | S | S | S | S | S | R | R |
| **PSA10662** | S | S | NT | S | NT | S | NT | S | S | NT | S | S | S | S | S | S |

Antibiotic susceptibilities are shown as follows: **S susceptible, R Resistant, I Intermediate, Ind indeterminate,** i.e there was no agreement between the two independent methods used**, NT Not tested**

**Supplemental Figures:**

**Fig S1: Growth curves panel strains.** The growth of the 42 panel strains in MHB at 37°C was monitored using the Bioscreen C to measure culture optical density over 48 hours. Each experiment was performed twice with four technical replicates per strain, per experiment.

**Fig. S2. Swimming, swarming and twitching motilities.** Representative images of the levels of swimming, swarming on LB agar, swarming on BSM-G agar and twitching motility exhibited by panel strains.

**Fig. S3. Alginate production.** a) Alginate production from cells grown in LB. b) Comparison of alginate production of selected mucoid and non-mucoid strains grown in LB agar (black bars) or PIA (grey bars). The results presented are the means ± SD of at least two independent analyses using at least two independent cultures of the same strain.

**Fig. S4. Urease Activity.** Urease activity of *Pseudomonas aeruginosa* strains on Christensen broth in the presence of urea. Results represent the means of three independent experiments ± SD.

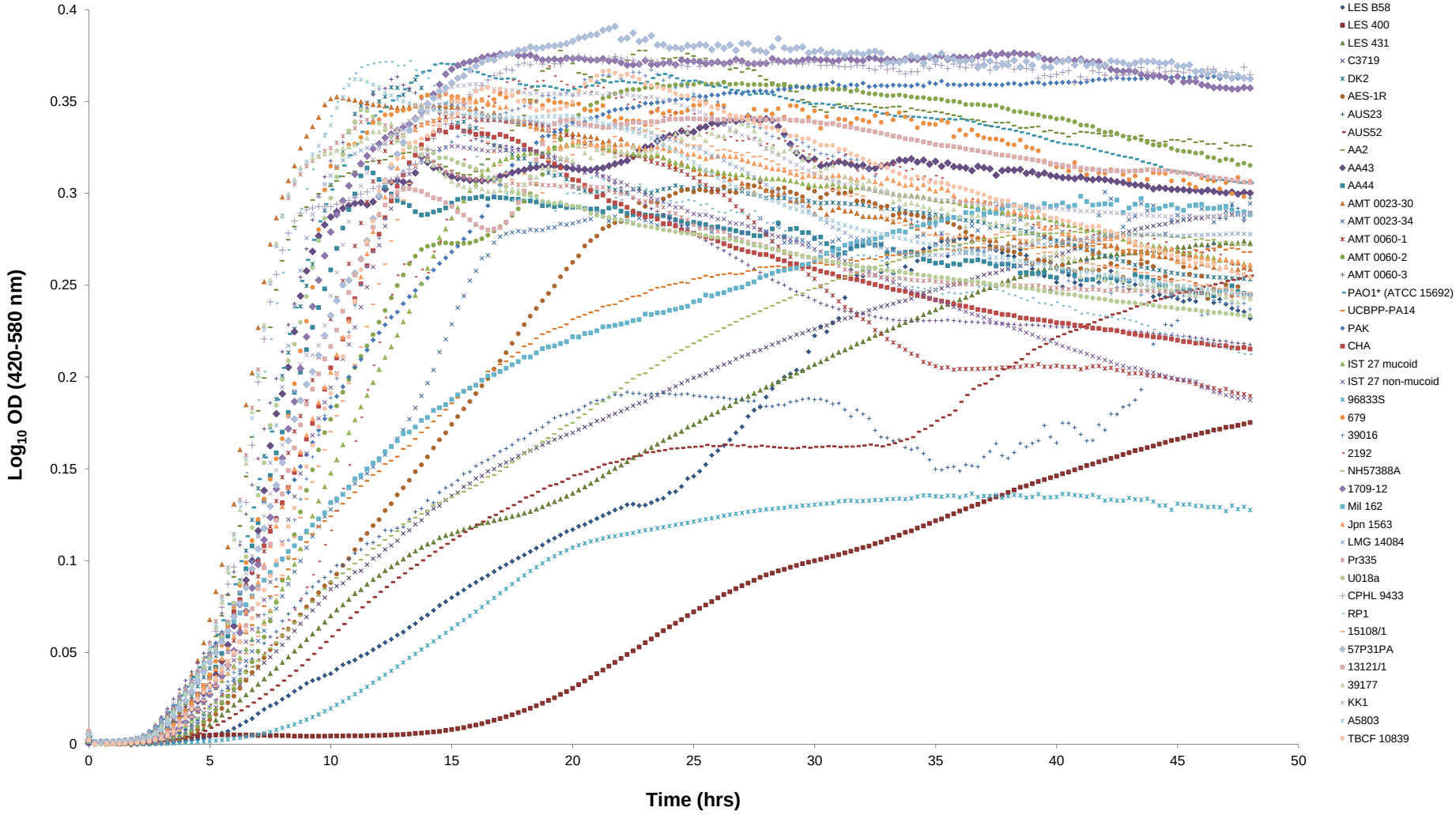

Log10 OD (420-580 nm)
Time (hrs)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0
5
10
15
20
25
30
35
40
45
50
LES B58
LES 400
LES 431
C3719
DK2
AES-1R
AUS23
AUS52
AA2
AA43
AA44
AMT 0023-30
AMT 0023-34
AMT 0060-1
AMT 0060-2
AMT 0060-3
PAO1* (ATCC 15692)
UCBPP-PA14
PAK
CHA
IST 27 mucoid
IST 27 non-mucoid
96833S
679
39016
2192
NH57388A
1709-12
Mil 162
Jpn 1563
LMG 14084
Pr335
U018a
CPHL 9433
RP1
15108/1
57P31PA
13121/1
39177
KK1
A5803
TBCF 10839

**Figure S1.**

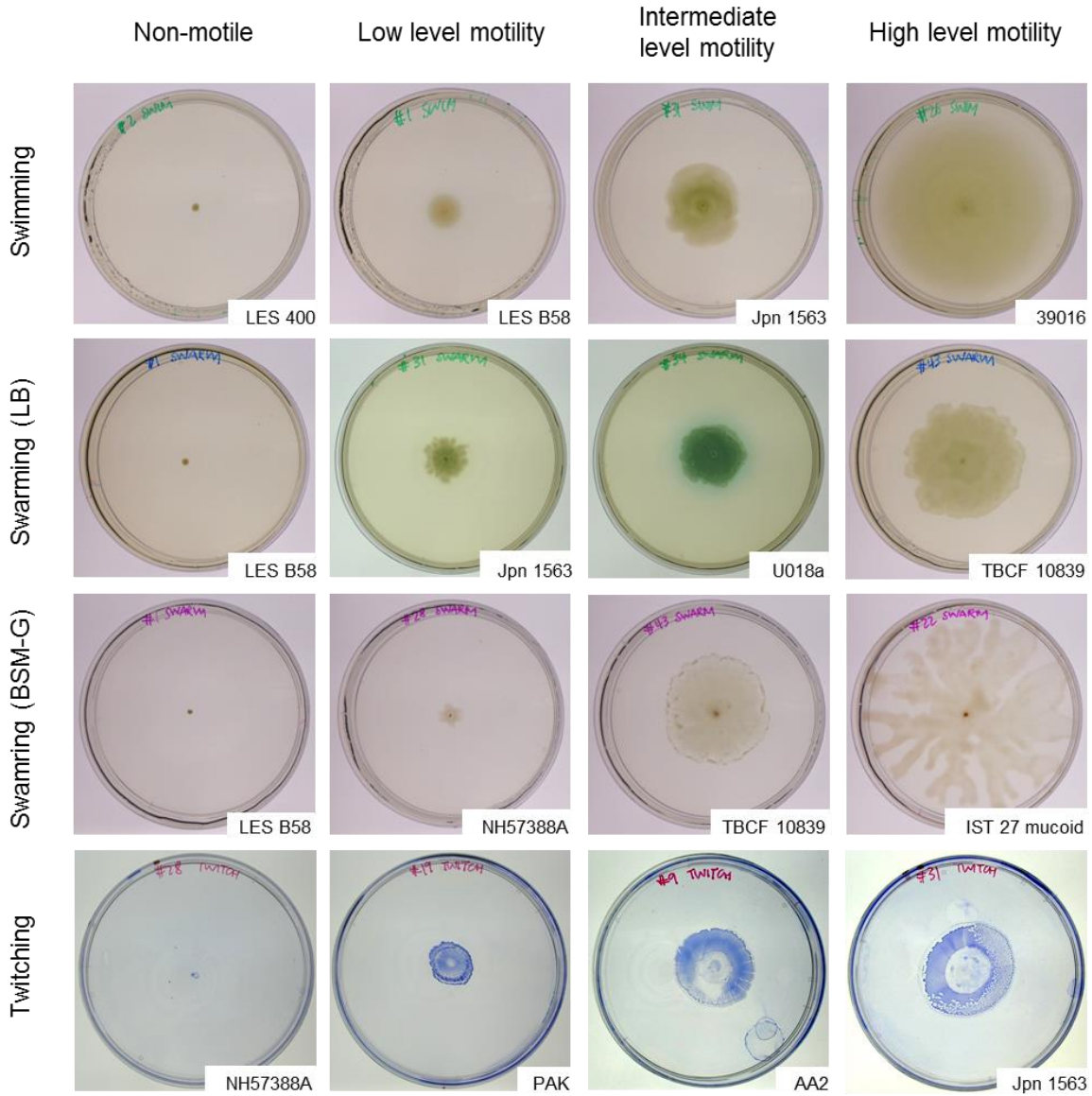
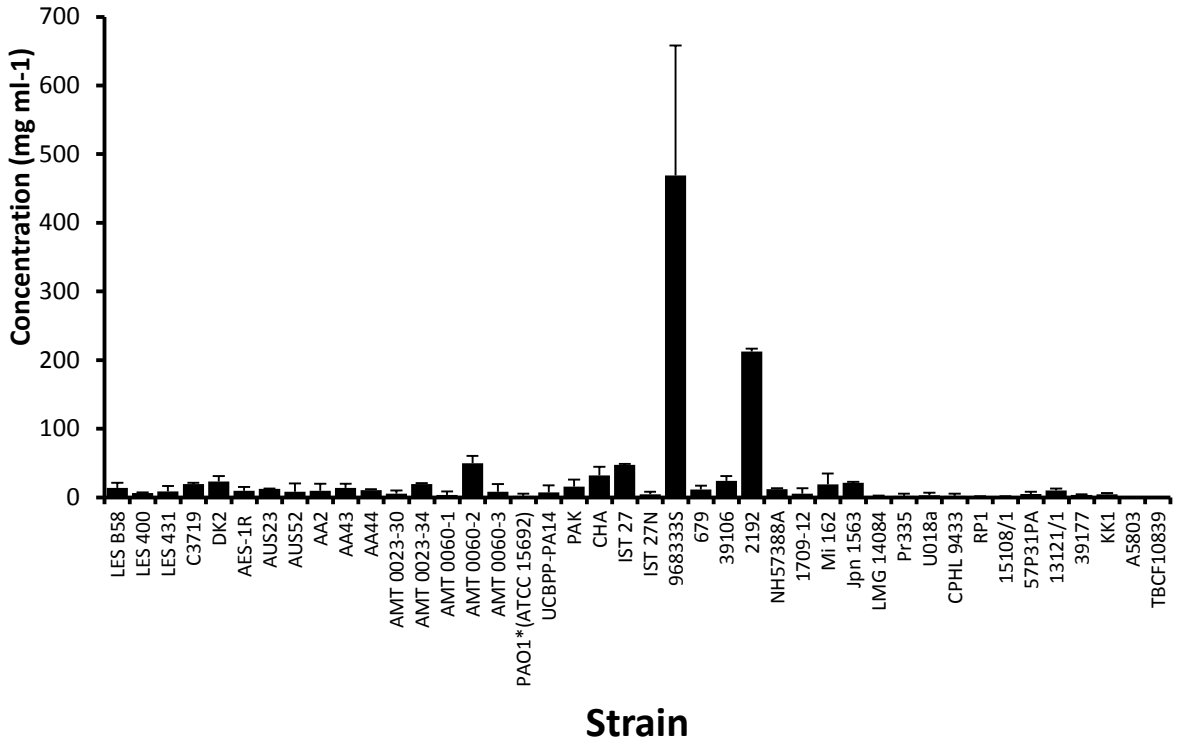

Non-motile
Low level motility
Intermediate level motility
High level motility
Swimming
Swarming (LB)
Swamring (BSM-G)
Twitching
LES 400
LES B58
Jpn 1563
39016
LES B58
Jpn 1563
U018a
TBCF 10839
LES B58
NH57388A
TBCF 10839
IST 27 mucoid
NH57388A
PAK
AA2
Jpn 1563

**Figure S2**

**Fig. 3(a)**


Concentration (mg ml-1)
700
600
500
400
300
200
100
0
LES B58
LES 400
LES 431
C3719
DK2
AES-1R
AUS23
AUS52
AA2
AA43
AA44
AMT 0023-30
AMT 0023-34
AMT 0060-1
AMT 0060-2
AMT 0060-3
PAO1*(ATCC 15692)
UCBPP-PA14
PAK
CHA
IST 27
IST 27N
968333S
679
39106
2192
NH57388A
1709-12
Mi 162
Jpn 1563
LMG 14084
Pr335
U018a
CPHL 9433
RP1
15108/1
57P31PA
13121/1
39177
KK1
A5803
TBCF10839
Strain

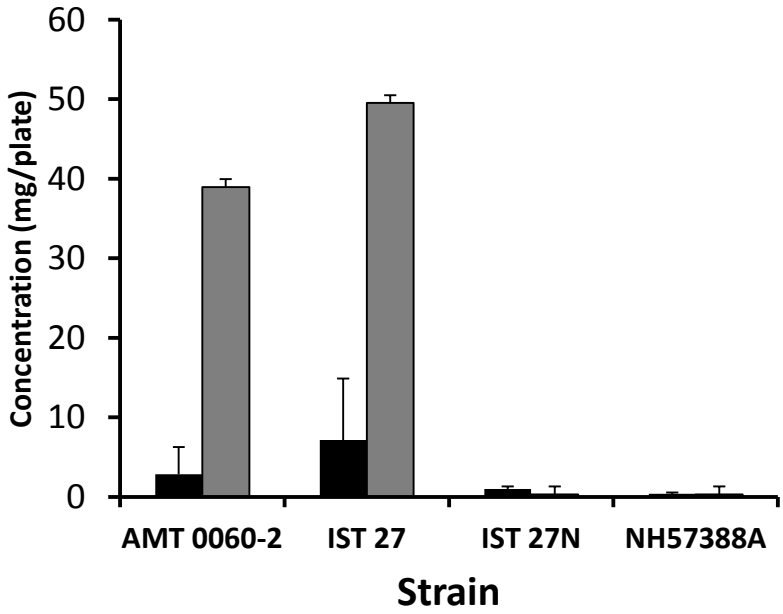
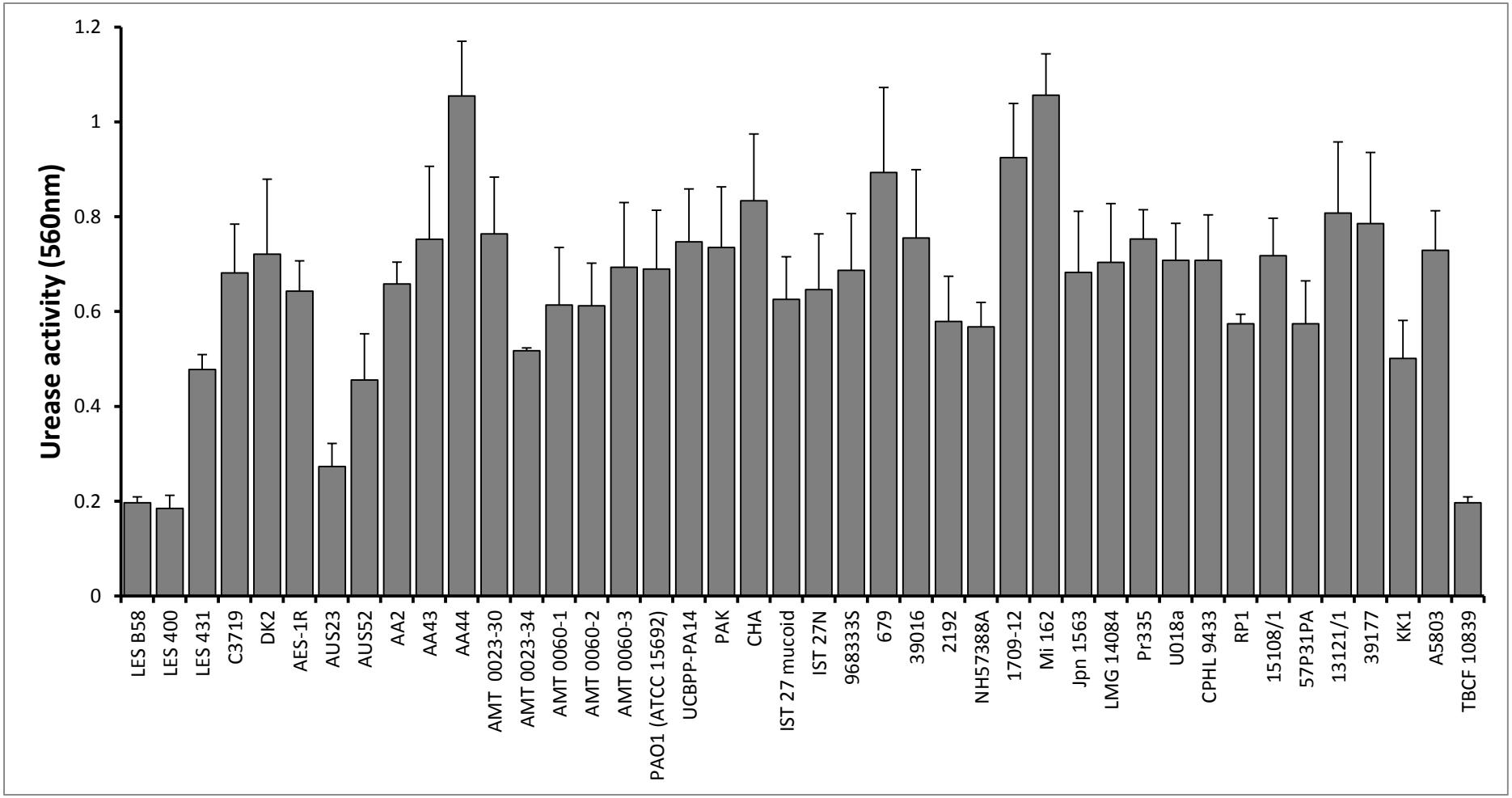

Concentration (mg/plate)
60
50
40
30
20
10
0
AMT 0060-2
IST 27
IST 27N
NH57388A
Strain

**Fig. 3(b)**

**Figure S4**


Urease activity (560nm)
1.2
1
0.8
0.6
0.4
0.2
0
LES B58
LES 400
LES 431
C3719
DK2
AES-1R
AUS23
AUS52
AA2
AA43
AA44
AMT 0023-30
AMT 0023-34
AMT 0060-1
AMT 0060-2
AMT 0060-3
PAO1 (ATCC 15692)
UCBPP-PA14
PAK
CHA
IST 27 mucoid
IST 27N
968333S
679
39016
2192
NH57388A
1709-12
Mi 162
Jpn 1563
LMG 14084
Pr335
U018a
CPHL 9433
RP1
15108/1
57P31PA
13121/1
39177
KK1
A5803
TBCF 10839