

Table 1- Source of samples

Hospital	Isolate ID	Source
DMC	27b	Urine
DMC	8b	Wound Swab
DMC	23b	Tracheal Aspirate
DMC	15b	Pus
DMC	54d	Wound Swab
DMC	20b	Wound Swab
DMC	20c	Wound Swab
DMC	7	Pus
DMC	28c	Wound Swab
DMC	30b	Pus
DMC	20d	Wound Swab
DMC	44	Pus
DMC	24	Wound Swab
BIHS	b01	Wound Swab
BIHS	b02	Wound Swab
BIHS	b03	Pus
BIHS	b04	Wound Swab
BIHS	b05	Wound Swab
BIHS	b06	Wound Swab
BIHS	b07	Wound Swab

Table 2 Primers used in this study for the detection of biofilm associated genes in *Pseudomonas aeruginosa*

Primer	Sequence (5'->3')	Tm	Length (bp)	Amplicon size	Reference
<i>PilT_F_</i>	TCCACGAGTCGAAGAAGTGC	60	20	145	This study
<i>PilT_R</i>	AGGCGAATGGTTTCCAGGTC	60	20		
<i>lecB_F</i>	CAAGGAGTGTTCAACCCTTCC	57	20	306	This study
<i>lecB_R</i>	GTCGTTGTAGTCGTTGTCGG	57	20		
<i>PelB_F</i>	ACGCCTGCTCTGGTTCTAC	58	19	186	This study
<i>PelB_R</i>	TTGGGATTGGACTTGAGGTA	58	20		
<i>RhlB_F</i>	CGCTGCTTGTCGTAATCCAC	59	20	96	This study
<i>RhlB_R</i>	GGCCATCCAGATCCACAAGG	60	20		

	27b (SBF)	20C (MBF)	30b(WBF)
Size	6,952,319	7,018,827	7,211,991
GC Content	65.8	65.9	65.7
Number of Subsystems	432	429	432
Number of Coding Sequences	7552	7112	7565
Number of RNAs	65	66	66
Pathogen Probability	0.727	0.751	0.749
MLST ST	New	664	244
Predicted strain	E6130952	PABL012	W16401

Table 3- Overview of the genomic profile of 3 *Pseudomonas aeruginosa* isolates with pathogen probability, MLST profiling and Kmer analysis.

anti

SMASH

antibiotics & Secondary Metabolite Analysis SShell

Version 4.2.0

Select Gene Cluster:

Overview

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Identified secondary metabolite clusters

Cluster	Type
The following clusters are from record c00015_NODE_15.. (original name was: NODE_15_length_58608_cov_34.9588_ID_17649...):	
Cluster 1	Other
The following clusters are from record c00030_NODE_30.. (original name was: NODE_30_length_45567_cov_34.9644_ID_17679...):	
Cluster 2	Hserlactone
The following clusters are from record c00044_NODE_44.. (original name was: NODE_44_length_35733_cov_33.1371_ID_17707...):	
Cluster 3	Phenazine
The following clusters are from record c00046_NODE_46.. (original name was: NODE_46_length_34972_cov_39.4119_ID_17711...):	
Cluster 4	Hserlactone
The following clusters are from record c00054_NODE_54.. (original name was: NODE_54_length_30565_cov_43.6816_ID_17727...):	
Cluster 5	Other
The following clusters are from record c00072_NODE_72.. (original name was: NODE_72_length_25509_cov_34.5039_ID_17763...):	
Cluster 6	Nrps
The following clusters are from record c00075_NODE_75.. (original name was: NODE_75_length_25000_cov_35.539_ID_17769...):	

Metabolites	27b	20c	30b
Homoserine lactone	+	+	+
Phenazine	+	+	+
Bacteriocin	+	+	+
Non ribosomal peptides	+	+	+
Arylpoleyene	-	-	+
Mangotoxin	-	-	71%
Pyocyanin	-	-	42%
Pyochelin	40%	40%	-

Table 4: Secondary metabolite profile of the 3 isolates.

**Table-5 Comparison of Biofilm related gene products of 3 *Pseudomonas aeruginosa* strains
(in comparison with reference strain *Pseudomonas aeruginosa* PA01)**

Name of the Genes/ Gene Products	Identity DMC-27b	Identity DMC-30b	Identity DMC-20c
<i>pilI</i> ; twitching motility protein PilI	100	100	100
<i>pilJ</i> ; twitching motility protein PilJ	99	100	99
<i>pilH</i> ; twitching motility protein PilH	100	100	100
<i>pilG</i> ; pilus biosynthesis/twitching motility protein PilG	100	100	100
<i>chpA</i> ; chemotactic signal transduction system protein	98	99	99
<i>chpC</i> ; chemotaxis protein	98	98	98
<i>cyaB</i> ; protein CyaB	99	100	100
<i>cpdA</i> ; cAMP phosphodiesterase	98	99	99
<i>vfr</i> ; cAMP-regulatory protein	100	100	100
<i>fleQ</i> ; transcriptional regulator FleQ	100	100	100
<i>exsA</i> ; exoenzyme S transcriptional regulator ExsA	100	100	100
<i>phnB</i> ; anthranilate synthase component II	99	99	99
<i>pqsA</i> ; anthranilate--CoA ligase	99	100	100
<i>pqsB</i> ; hypothetical protein	98	100	99
<i>pqsC</i> ; hypothetical protein	99	99	100
<i>pqsD</i> ; 3-oxoacyl-ACP synthase	99	99	99
<i>pqsE</i> ; thioesterase PqsE	100	100	100
<i>pqsH</i> ;	100	100	100
<i>mvfR</i> ; transcriptional regulator MvfR	99	99	99
<i>rhlI</i> ; acyl-homoserine-lactone synthase	99	99	99
<i>rhlR</i> ; transcriptional regulator RhlR	100	100	100
<i>rhlA</i> ; rhamnosyltransferase subunit A	99	99	98
<i>rhlB</i> ; rhamnosyltransferase subunit B	100	100	99
<i>rhlC</i> ; rhamnosyltransferase	100	99	99
<i>lecA</i> ; PA-I galactophilic lectin	99	100	99
fucose-binding lectin PA-IIL, <i>LecB</i>	87	100	100
sensor histidine kinase <i>SagS</i>	99	100	100
hybrid sensor kinase, PA1611	99	99	99
<i>ercS</i> '; sensor histidine kinase	99	99	99
histidine phosphotransfer protein <i>HptB</i>	99	99	99
biofilm regulator <i>HsbR</i>	99	100	100
anti anti-sigma factor <i>HsbA</i>	100	100	100
<i>gacA</i> ; response regulator <i>GacA</i>	100	100	100
<i>gacS</i> ; sensor/response regulator hybrid protein	99	100	100
<i>retS</i> ; sensor histidine kinase <i>MifS</i>	99	99	99
<i>rsmY</i> ; regulatory RNA <i>RsmY</i>	100	100	100
<i>rsmZ</i> ; regulatory RNA <i>RsmZ</i>	100	100	100

<i>rsmA</i> ; carbon storage regulator	100	100	100
<i>ppkA</i> ; serine/threonine protein kinase PpkA	99	99	98
<i>wspD</i> ; hypothetical protein	100	100	100
<i>wspA</i> ; chemotaxis transducer	98	99	99
<i>wspE</i> ; chemotaxis sensor/effector fusion protein	99	99	100
probable methylesterase, WspF	100	100	99
<i>wspR</i> ; two-component response regulator	99	100	100
<i>sadC</i> , protein SadC	99	100	100
<i>siaD</i> , protein SiaD	99	99	99
diguanylate cyclase, TpbB	99	99	99
<i>roeA</i> , Protein RoeA	98	100	98
<i>mucR</i> ; signaling protein	99	99	100
<i>bifA</i> ; protein BifA	100	100	100
transcriptional regulator, FleQ	100	100	100
<i>pslB</i> ; biofilm formation protein PslB	99	99	99
<i>pslC</i> ; biofilm formation protein PslC	99	99	99
<i>pslD</i> ; biofilm formation protein PslD	100	100	100
<i>pslE</i> ; biofilm formation protein PslE	99	99	99
<i>pslF</i> ; biofilm formation protein PslF	99	99	99
<i>pslG</i> ; biofilm formation protein PslG	100	100	100
<i>pslH</i> ; biofilm formation protein PslH	99	99	99
<i>pslI</i> ; biofilm formation protein PslI	100	99	99
<i>pslJ</i> ; biofilm formation protein PslJ	100	100	100
<i>pslK</i> ; biofilm formation protein PslK	98	99	99
<i>pslL</i> ; hypothetical protein	99	100	99
<i>pslM</i> ; biofilm formation protein PslM	99	99	99
<i>pslN</i> ; biofilm formation protein PslN	99	100	99
<i>pelA</i> ; hypothetical protein	99	93	99
<i>pelB</i> ; pellicle/biofilm biosynthesis protein PelB	98	87	99
<i>pelC</i> ; pellicle/biofilm biosynthesis protein PelC	100	95	100
<i>pelD</i> ; pellicle/biofilm biosynthesis protein PelD	98	90	98
<i>pelE</i> ; pellicle/biofilm biosynthesis protein PelE	97	90	99
<i>pelF</i> ; pellicle/biofilm biosynthesis glycosyltransferase PelF	99	90	99
<i>pelG</i> ; pellicle/biofilm biosynthesis transporter PelG	100	96	100
alginate biosynthesis protein, Alg44	100	100	100
<i>trpE</i> ; anthranilate synthase component I	99	99	99
anthranilate synthetase component II, <i>trpG</i>	98	98	98
<i>flgM</i> ; protein FlgM	100	99	100
<i>fliA</i> ; flagellar biosynthesis sigma factor FliA	100	100	100
<i>lasI</i> ; acyl-homoserine-lactone synthase	100	100	100
<i>lasR</i> ; transcriptional regulator LasR	98	100	100
<i>pppA</i> ; serine/threonine phosphatase PppA	99	99	99
<i>stp1</i> ; phosphatase Stp1	100	100	100
<i>clpV1</i> ; secretion protein ClpV1	99	99	99

<i>hcpA</i> ; secreted protein Hcp	100	100	100
<i>hcp1</i> ; protein secretion apparatus assembly protein	100	100	100
<i>icmF1</i> ; type VI secretion protein IcmF	99	100	99
<i>phnA</i> ; anthranilate synthase component I	98	98	98
<i>pslA</i> ; biofilm formation protein PslA	100	99	100
<i>algA</i> ; isomerase	100	99	100
<i>fha1</i> ; Fha domain-containing protein	99	98	99
<i>ladS</i> ; lost adherence sensor LadS	99	98	99

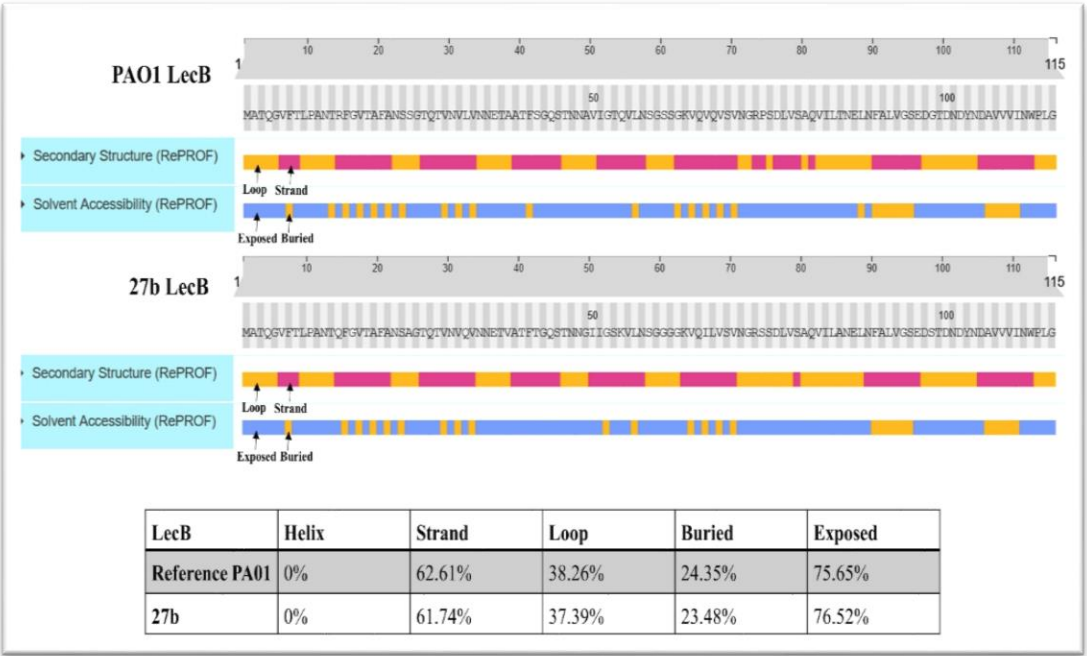


Figure 1: Secondary structure and Solvent Accessibility analysis of 27b LecB protein and Reference PAO1 LecB protein.

30b <i>pelA</i>	Description ▼	Query Cover ▼	E value ▼	Per. Ident ▼	Accession
	Pseudomonas aeruginosa strain FDAARGOS_570 chromosome, complete...	100%	0.0	100.00%	CP033835.1
	Pseudomonas aeruginosa PA7, complete genome	100%	0.0	99.82%	CP000744.1
	Pseudomonas aeruginosa strain CR1 chromosome, complete genome	100%	0.0	99.36%	CP020560.1
	Pseudomonas aeruginosa strain AR_0356 chromosome, complete genome	100%	0.0	99.36%	CP027169.1
	Pseudomonas aeruginosa strain AR441 chromosome, complete genome	100%	0.0	99.33%	CP029093.1
	Pseudomonas aeruginosa strain AZPAE15042 chromosome, complete gen...	100%	0.0	99.26%	CP041354.1
	Pseudomonas aeruginosa strain PA-3 chromosome, complete genome	96%	0.0	92.83%	CP033084.1
	Pseudomonas aeruginosa strain LW chromosome, complete genome	96%	0.0	92.79%	CP022478.1
	Pseudomonas aeruginosa PA96 genome	96%	0.0	92.79%	CP007224.1
	Pseudomonas aeruginosa strain FDAARGOS_505 chromosome, complete...	95%	0.0	92.93%	CP033832.1

Organism	Accession	<i>pelA</i> identity	<i>pelB</i> identity	<i>pelC</i> identity	<i>pelD</i> identity	<i>pelE</i> identity	<i>pelF</i> identity	<i>pelG</i> identity
<i>Pseudomonas aeruginosa</i> strain FDAARGOS_570	CP033835.1	100	100	100	100	100	100	100
<i>Pseudomonas aeruginosa</i> PA7	CP000744.1	99.82	99.78	99.81	99.85	99.8	99.93	99.85
<i>Pseudomonas aeruginosa</i> strain CR1	CP020560.1	99.36	99.58	99.61	98.46	99.7	98.56	99.49
<i>Pseudomonas aeruginosa</i> strain AR_0356	CP027169.1	99.36	99.53	99.61	98.61	99.6	98.56	99.2
<i>Pseudomonas aeruginosa</i> strain AR441	CP029093.1	99.33	99.53	99.61	98.61	99.6	98.49	99.2
<i>Pseudomonas aeruginosa</i> strain AZPAE15042	CP041354.1	99.26	99.64	99.81	98.54	99.6	98.69	99.49

Table 6: NCBI nucleotide blast result for 30b *pelA*. The blue color box shows the strains that have 99-100% sequence homology with 30b *pelA*. Seven Pel operon genes of six *Pseudomonas*

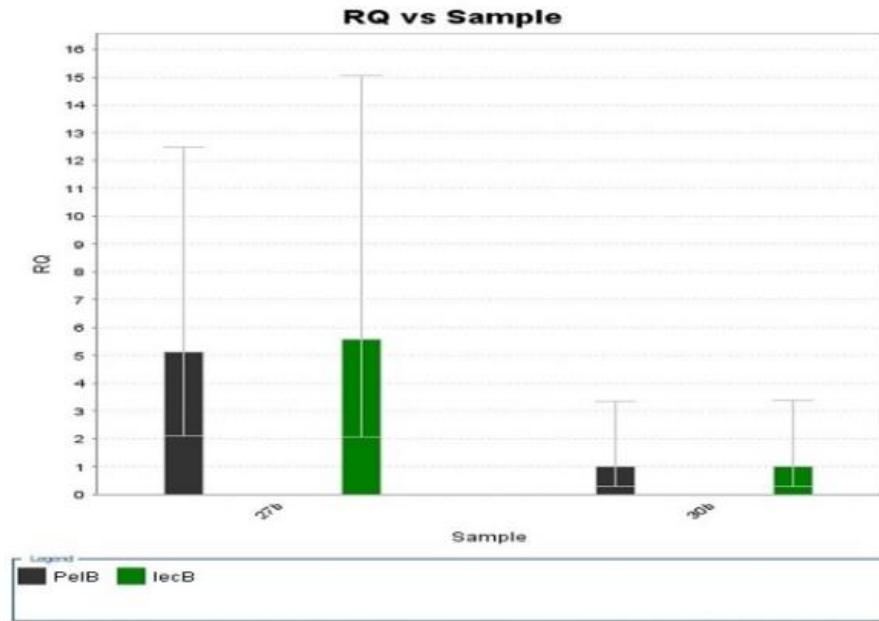


Fig 4: Relative quantification of *pelB* (black bars) and *lecB* (green bars) transcript levels after 24 hours in 27b and 30b.

Table 6: RQ data table representing targets, $\Delta\Delta C_t$ and RQ values

<i>Sample</i>	<i>Target</i>	$\Delta\Delta C_t$	<i>RQ value</i>
27b_24hr	pelB	-2.355	5.116
30b_24hr	pelB	0	1
27b_24hr	lecB	-2.483	5.59
30b_24hr	lecB	0	1