

Supplementary Materials

***In silico* analysis reveals novel class of quorum sensing genes and consistent presence of LuxR solos in the *Pandoraea* species**

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Supplementary Table S1. All bacteria strains with their description and culturing conditions used in this study.

Strain	Description	Culturing media, temperature	Source/reference
<i>Chromobacterium violaceum</i> CV026	Biosensor derived from double mini-Tn5 mutant of ATCC31532 that synthesize purple violacein pigment for detection of short chain exogenous AHL.	LBA, 28°C	(McClellan et al., 1997)
<i>Erwinia carotovora</i> GS101	Produces short chain exogenous AHL which served as positive control in CVO26 bioassay.	LBA, 28°C	Gift from Prof. Paul Williams
<i>Erwinia carotovora</i> PNP22	Negative control for CVO26 bioassay.	LBA, 28°C	Gift from Prof. Paul Williams
<i>Pandoraea pnomenusa</i> RB-38	Soil isolate	LBA, 28°C	This study
<i>Pandoraea pnomenusa</i> RB-44	Soil isolate	LBA, 28°C	This study
<i>Pandoraea pnomenusa</i> 3kgm	Soil isolate	LBA, 28°C	This study
<i>Pandoraea pnomenusa</i> DSM 16536 ^T	Recovered from cystic fibrosis patient, United Kingdom.	TSA, 37°C	(Coenye et al., 2000)
<i>Pandoraea pnomenusa</i> 6399	Recovered 11 months before the cystic fibrosis patient deceased, Australia.	TSA, 37°C	(Ee et al., 2015a)
<i>Pandoraea pnomenusa</i> 7641	Recovered from deceased cystic fibrosis patient, Australia.	TSA, 37°C	(Ee et al., 2015b)
<i>Pandoraea pulmonicola</i> DSM 16583 ^T	Recovered from sputum of cystic fibrosis patient, Canada.	TSA, 37°C	(Coenye et al., 2000)
<i>Pandoraea sputorum</i> DSM 21091 ^T	Recovered from sputum of cystic fibrosis patient USA.	TSA, 37°C	(Coenye et al., 2000)
<i>Pandoraea apista</i> DSM 16535 ^T	Recovered from sputum of cystic fibrosis patient, Denmark.	TSA, 37°C	(Coenye et al., 2000)
<i>Pandoraea faecigallinarum</i> DSM 23572 ^T	Isolated from chicken dung, India.	TSA, 28°C	(Sahin et al., 2011)
<i>Pandoraea norimbergensis</i> DSM 11628 ^T	Isolated from oxic water above sulfide containing lake sediment, Germany.	TSA, 37°C	(Coenye et al., 2000)
<i>Pandoraea thiooxydans</i> DSM 25325 ^T	Isolated from rhizosphere soils of sesame <i>Sesamum indicum</i> L., Korea.	TSA, 30°C	(Anandham et al., 2010)
<i>Pandoraea vervacti</i> DSM 23571 ^T	Isolated from uncultivated field soil, Turkey	TSA, 28°C	(Sahin et al., 2011)
<i>Pandoraea oxalativorans</i> DSM 23570 ^T	Isolated from soil litter close to <i>Oxalis</i> sp., Turkey.	TSA, 28°C	(Sahin et al., 2011)

Abbreviation:

LBA, Luria-bertani agar;

TSA, Tryptic soy agar

Supplementary Table 2. Precursor and product ion m/z values of synthetic AHLs.

Synthetic AHL	Precursor ion, Q1 (m/z)	Product ion, Q3 (m/z)
C4-HSL	172	102
C6-HSL	200	102
C7-HSL	214	102
C8-HSL	228	102
C10-HSL	256	102
C12-HSL	284	102
3-oxo-C6-HSL	214	102
3-oxo-C8-HSL	242	102
3-oxo-C10-HSL	270	102
3-oxo-C12-HSL	298	102

Supplementary Table 3. Designation code and accession number of all *Pandoraea* genomes retrieved from GenBank for this study.

Strain	Designation code	Finishing status	GenBank ID
<i>P. pnomenusa</i> RB-38	Ppn RB38	Complete genome	CP007506.3
<i>P. pnomenusa</i> RB-44	Ppn RB44	Complete genome	CP006938.1
<i>P. pnomenusa</i> 3kgm	Ppn 3kgm	Complete genome	CP006900.2
<i>P. pnomenusa</i> 6399	Ppn 6399	Draft genome	JTCR01
<i>P. pnomenusa</i> 7641	Ppn 7641	Draft genome	JTCS01
<i>P. apista</i> AU2161	Pap AU21	Complete genome	CP011501.1
<i>P. apista</i> TF80G25	Pap TF80	Complete genome	CP011279.1
<i>P. apista</i> TF81F4	Pap TF81	Complete genome	CP010518.3
<i>Pandoraea</i> sp. B-6	B-6	Draft genome	AKXS01
<i>Pandoraea</i> sp. E26	E26	Draft genome	AYXJ01
<i>Pandoraea</i> sp. SD6-2	SD6-2	Draft genome	AQOU01

Supplementary Table 4. ANI analysis between genomes of *Pandoraea* species.

		Ppn						Ppu	Psp	Pap				Pno	Pfa	Pox	Pth	Pve	<i>Pandoraea</i> sp.		
		DSM	RB38	RB44	3kgm	6399	7641			DSM	DSM	DSM	AU21						TF80	TF81	DSM
Ppn	DSM	100	99.32	99.34	99.31	85.04	85.14	85.49	84.50	84.55	84.469	84.50	84.55	83.92	84.66	84.61	78.62	84.61	84.81	99.35	84.65
	RB38	99.32	100	99.41	99.44	84.66	84.59	85.06	84.50	84.43	84.48	84.46	84.40	83.97	84.52	84.71	78.57	84.67	84.77	99.41	84.67
	RB44	99.34	99.41	100	99.43	84.64	84.63	85.07	84.48	84.51	84.47	84.46	84.46	83.96	84.49	84.63	78.68	84.61	84.79	99.97	84.61
	3kgm	99.31	99.44	99.43	100	84.61	84.58	85.07	84.43	84.47	84.48	84.45	84.49	83.94	84.54	84.64	78.54	84.67	84.85	99.44	84.67
	6399	85.04	84.66	84.64	84.61	100	100	84.78	84.66	88.56	88.55	88.51	88.55	84.14	84.66	84.78	78.14	84.60	85.49	84.61	87.89
	7641	85.14	84.59	84.63	84.58	100	100	84.77	84.64	88.46	88.55	88.53	88.48	84.12	84.58	84.79	78.21	84.60	85.45	84.65	87.86
Ppu		85.49	85.06	85.07	85.07	84.78	84.77	100	84.68	84.60	84.55	84.58	84.60	84.33	84.63	84.80	78.79	84.73	85.05	85.10	84.75
Psp		84.50	84.50	84.48	84.43	84.66	84.64	84.68	100	84.55	84.54	84.64	84.55	83.80	85.85	92.39	78.37	85.56	84.60	84.41	84.71
Pap	DSM	84.55	84.43	84.51	84.47	88.56	88.46	84.60	84.55	100	99.31	99.31	99.31	84.15	84.58	84.68	78.19	84.44	85.32	84.44	87.77
	AU21	84.46	84.48	84.47	84.48	88.55	88.55	84.55	84.54	99.31	100	100	100	84.10	84.46	84.59	78.11	84.42	85.32	84.42	87.78
	TF80	84.50	84.46	84.46	84.45	88.51	88.53	84.58	84.64	99.31	100	100	100	84.10	84.48	84.72	78.11	84.48	84.30	84.48	87.77
	TF81	84.55	84.40	84.46	84.49	88.55	88.48	84.60	84.55	99.31	100	100	100	84.15	84.50	84.68	78.19	84.44	85.29	84.41	87.74
Pno		83.92	83.97	83.96	83.94	84.14	84.12	84.33	83.80	84.15	84.10	84.10	84.15	100	83.88	83.99	78.24	83.90	84.09	83.92	84.05
Pfa		84.66	84.52	84.49	84.54	84.66	84.58	84.63	85.80	84.58	84.46	84.48	84.50	83.80	100	85.10	78.47	86.70	84.54	84.47	84.62
Pox		84.61	84.71	84.63	84.64	84.78	84.79	84.80	92.39	84.68	84.59	84.72	84.68	83.99	85.72	100	78.35	85.90	84.71	84.60	84.79
Pth		78.62	78.57	78.68	78.54	78.14	78.21	78.79	78.37	78.19	78.11	78.11	78.19	78.24	78.47	78.35	100	78.25	78.08	78.42	77.85
Pve		84.61	84.67	84.61	84.67	84.60	84.60	84.73	85.56	84.44	84.42	84.48	84.44	83.90	86.70	85.90	78.25	100	84.53	84.49	84.59
sp.	B-6	84.81	84.77	84.79	84.85	85.49	85.45	85.05	84.60	85.32	85.32	84.30	85.29	84.09	84.54	84.71	78.08	84.53	100	85.05	85.81
	E26	99.35	99.41	99.97	99.44	84.61	84.65	85.10	84.41	84.44	84.42	84.48	84.41	83.92	84.47	84.60	78.42	84.49	85.05	100	84.67
	SD6-2	84.65	84.67	84.61	84.67	87.89	87.86	84.75	84.71	87.77	87.78	87.77	87.74	84.05	84.62	84.79	77.85	84.59	85.81	84.67	100

Supplementary Table 5. Gene designation, accession number, amino acid length, GC content and genetic orientation of all canonical LuxI/R1 and LuxR solos of *Pandora* species.

Strain	CVO26 bioassay (AHL detected)	Canonical LuxI Accession number (Length/GC content) Genetic orientation	Canonical LuxR1 Accession number (Length/GC content) Genetic orientation	LuxR2 solo Accession number (Length/GC content) Genetic orientation	LuxR3 solo Accession number (Length/GC content) Genetic orientation
Ppn DSM	Positive (C8-HSL)	PpnI ALR35832.1 (245aa / 65.4%) →	PpnR1 ALR35833.1 (233aa / 62.0%) ←	PpnR2 AIU26521.1 (255aa / 61.1%) →	PpnR3 CP009553.2 (4877704bp - 4878696bp) (330aa / 67.4%) →
Ppn RB38	Positive (C8-HSL)	PpnI AHN77101.1 (261aa / 65.5%) →	PpnR1 AHN77102.1 (233aa / 62.0%) ←	PpnR2 AHN76935.1 (255aa / 61.0%) →	PpnR3 CP007506.2 (4853186bp - 4854178bp) (330aa / 67.3%) →
Ppn RB44	Positive (C8-HSL)	PpnI AHB74553.1 (261aa / 65.5%) →	PpnR1 AHB74552.1 (233aa / 61.3%) ←	PpnR2 AHB74721.1 (255aa / 61.1%) →	PpnR3 CP006938.1 (2823449bp – 2824450bp) (333 aa / 67.5%) →
Ppn 3kgm	ND	PpnI WP_052167255.1 (191aa / 67.7%) →	PpnR1 WP_023594976.1 (233aa / 61.5%) ←	PpnR2 AHB04908.1 (255aa / 61.1%) →	PpnR3 AHB08670.1 (330aa / 67.3%) →
Ppn 6399	ND	-	-	PpnR2 KHQ92691.1 (255aa / 59.1%) →*	PpnR3 WP_052240333.1 (275aa / 65.0%) →*
Ppn 7641	ND	-	-	PpnR2 KHQ93427.1 (255aa / 59.1%) →*	PpnR3 (275 aa) WP_052240333.1 (275aa / 65.0%) →*
Ppu DSM	-	-	-	PpuR2 AJC19606.1 (255aa / 63.0%) →	PpuR3 WP_052266958.1 (271aa / 68.4%) →
Psp DSM	Positive (C8-HSL)	PspI WP_052252565.1	PspR1 AJC18748.1	PspR2 AJC18152.1	PspR3 AJC15852.1

		(251aa / 64.4%) →	(233aa / 57.3%) ←	(255aa / 60.4%) →	(304aa / 64.6%) →
Pap DSM	-	-	-	PapR2 ALS67327.1 (255aa / 57.9%) →	PapR3 ALS65841.1 (275aa / 63.9%) →
Pap AU21	ND	-	-	PapR2 AKI63579.1 (255aa / 57.9%) →	PapR3 WP_048628601.1 (275aa / 63.9%) →
Pap TF80	ND	-	-	PapR2 AKH71307.1 (255aa / 57.9%) →	PapR3 WP_048628601.1 (275aa / 63.9%) →
Pap TF81	ND	-	-	PapR2 AJE97338.1 (255aa / 57.9%) →	Not found
Pno DSM	Negative (ND)	PnoI ALS60799.1 (249aa / 64.8%) →	PnoR1 ALS60800.1 (237aa / 58.7%) ←	PnoR2 ALS58731.1 (275aa / 61.2%) →	PnoR3 ALS60193.1 (246aa / 63.4%) →
Pfa DSM	Negative (ND)	PfaI AKM29569.1 (248aa / 64.5%) →	PfaR1 AKM32510.1 (233aa / 58.1%) ←	PfaR2 AKM29693.1 (255aa / 60.3%) →	PfaR3 WP_053059535.1 (310aa / 68.5%) →
Pox DSM	Positive (C8-HSL)	PoxI AKC71607.1 (222aa / 60.7%) →	PoxR1 AKC72674.1 (233aa / 56.3%) ←	PoxR2 AKC71755.1 (255aa / 60.0%) →	PoxR3 AKC69307.1 (304aa / 65.2%) →
Pth DSM	-	-	-	-	-
Pve DSM	Positive (C8-HSL)	PveI AJP59868.1 (217aa / 62.8%) →	PveR1 AJP59867.1 (233aa / 59.4%) ←	PveR2 AJP58402.1 (255aa / 58.9%) →	PveR3 AJP56205.1 (286aa / 66.3%) →
B-6	ND	-	-	LuxR2 WP_017233913.1 (255aa / 61.6%) →*	LuxR3 WP_017232691.1 (275aa / 66.8%) →*

E26	ND	LuxI WP_051399213.1 (191aa / 68.1%) →	LuxR1 WP_051610646.1 (233aa / 61.4%) ←	LuxR2 WP_023594793.1 (255aa / 61.1%) →*	Not found
SD6-2	ND	-	-	LuxR2 EON12348.1 (255aa / 56.6%) →*	LuxR3 EON14461.1 (275aa / 63.9%) →*

Note:
 Asterisk (*) indicates for unreliable orientation of *luxR2* solo and *luxR3* solo that were retrieved from different contigs of a draft genome.

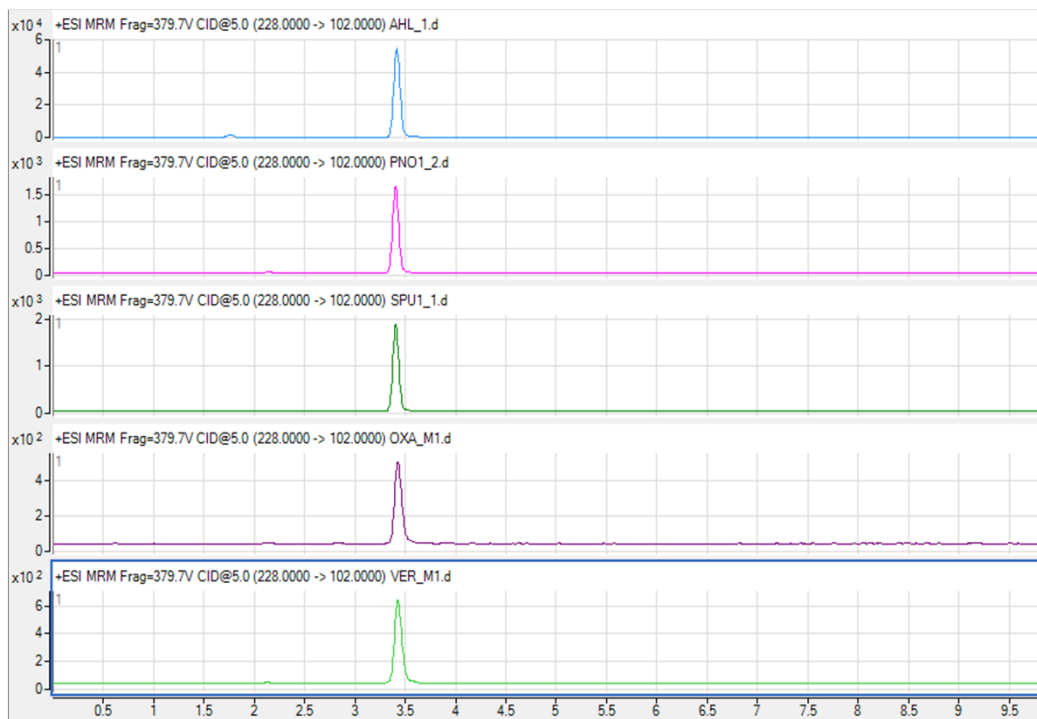
Supplementary Table 6. Multiple alignment analysis of LuxI of *Pandoraea* species. Numbering is relative to curated LuxI sequence of *Vibrio fischeri* (Entry: P12747) retrieved from Swiss-Prot database. Residues labelled with asterisk are residues whose mutation contributed significance loss in activity in LuxI and RhlI (Fuqua and Greenberg, 2002). PpnI 3kgm and LuxI E26 were retrieved from local PROKKA analysis as the sequences annotated by NCBI are truncated.

LuxI	Key amino acid conserved in all LuxI-type protein									
	R25*	F29	W35	E44*	D46*	D49*	R70*	F84	E101	R104*
PpnI DSM	R	F	W	E	D	D	R	F	E	R
PpnI RB38	R	F	W	E	D	D	R	F	E	R
PpnI RB44	R	F	W	E	D	D	R	F	E	R
PpnI 3kgm	R	F	W	E	D	D	R	F	E	R
PnoI DSM	R	F	W	E	D	D	R	F	E	R
PfaI DSM	R	F	W	E	D	D	R	F	E	R
PspI DSM	R	F	W	E	D	D	R	F	E	R
PoxI DSM	R	F	W	E	D	D	R	F	E	R
PveI DSM	R	F	W	E	D	D	R	F	E	R
LuxI E26	R	F	W	E	D	D	R	F	E	R

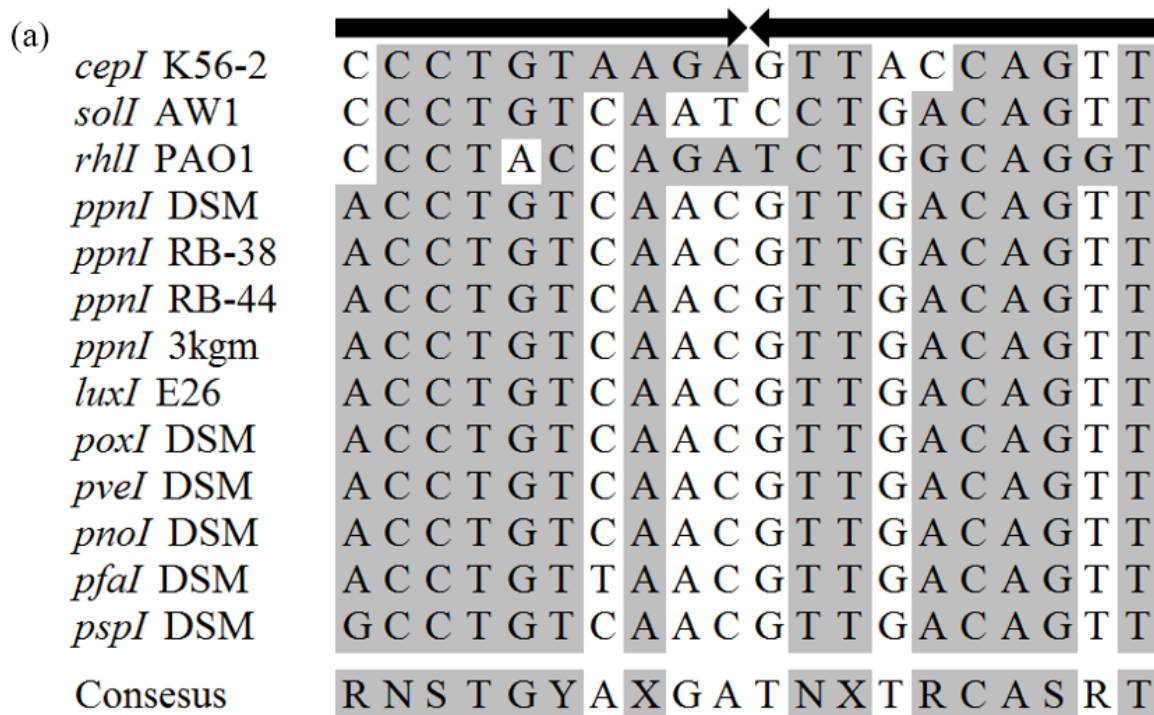
Supplementary Table 7. Multiple alignment analysis of LuxR of *Pandora* species. Numbering is relative to curated TraR sequence of *Agrobacterium tumefaciens* (Entry: P33905) retrieved from Swiss-Prot database (Subramoni et al., 2015).

LuxR		Key amino acid in autoinducer binding domain						Key amino acid in DNA binding domain		
		W57	Y61	D70	P71	W85	G113	E178	L182	G188
LuxR1	PpnR1 DSM	W	Y	D	P	W	G	E	L	G
	PpnR1 RB38	W	Y	D	P	W	G	E	L	G
	PpnR1 RB44	W	Y	D	P	W	G	E	L	G
	PpnR1 3kgm	W	Y	D	P	W	G	E	L	G
	PspR1 DSM	W	Y	D	P	W	G	E	L	G
	PnoR1 DSM	W	Y	D	P	W	G	E	L	G
	PfaR1 DSM	W	Y	D	P	W	G	E	L	G
	PoxR1 DSM	W	Y	D	P	W	G	E	L	G
	PveR1 DSM	W	Y	D	P	W	G	E	L	G
	LuxI E26	W	Y	D	P	W	G	E	L	G
LuxR2	PpnR2 DSM	W	Y	D	P	W	G	E	L	G
	PpnR2 RB38	W	Y	D	P	W	G	E	L	G
	PpnR2 RB44	W	Y	D	P	W	G	E	L	G
	PpnR2 3kgm	W	Y	D	P	W	G	E	L	G
	PpnR2 6399	W	Y	D	P	W	G	E	L	G
	PpnR2 7641	W	Y	D	P	W	G	E	L	G
	PpuR2 DSM	W	Y	D	P	W	G	E	L	G
	PspR2 DSM	W	Y	D	P	W	G	E	L	G
	PapR2 DSM	W	Y	D	P	W	G	E	L	G
	PapR2 AU21	W	Y	D	P	W	G	E	L	G
	PapR2 TF80	W	Y	D	P	W	G	E	L	G
	PapR2 TG81	W	Y	D	P	W	G	E	L	G
	PnoR2 DSM	W	Y	D	P	W	G	E	L	G
	PfaR2 DSM	W	Y	D	P	W	G	E	L	G
	PoxR2 DSM	W	Y	D	P	W	G	E	L	G
	PveR2 DSM	W	Y	D	P	W	G	E	L	G
	LuxR2 B-6	W	Y	D	P	W	G	E	L	G
	LuxR2 E26	W	Y	D	P	W	G	E	L	G
LuxR2 SD6-2	W	Y	D	P	W	G	E	L	G	
LuxR3	PpnR3 DSM	W	Y	D	P	W	G	E	L	G
	PpnR3 RB38	W	Y	D	P	W	G	E	L	G
	PpnR3 RB44	W	Y	D	P	W	G	E	L	G
	PpnR3 3kgm	W	Y	D	P	W	G	E	L	G
	PpnR3 6399	W	Y	D	P	W	G	E	L	G
	PpnR3 7641	W	Y	D	P	W	G	E	L	G
	PpuR3 DSM	W	Y	D	P	W	G	E	L	G
	PspR3 DSM	W	Y	D	P	W	G	E	L	G
	PapR3 DSM	W	Y	D	P	W	G	E	L	G
	PapR3 AU21	W	Y	D	P	W	G	E	L	G
	PapR3 TF80	W	Y	D	P	W	G	E	L	G
	PnoR3 DSM	W	Y	D	P	W	G	E	L	G
	PfaR3 DSM	W	Y	D	P	W	G	E	L	G
	PoxR3 DSM	W	Y	D	P	W	G	E	L	G
	PveR3 DSM	W	Y	D	P	W	G	E	L	G
	LuxR3 B-6	W	Y	D	P	W	G	E	L	G
	LuxR3 SD6-2	W	Y	D	P	W	G	E	L	G

Supplementary Figure 1. MRM MS analysis for characterization of AHL profile. Synthetic C8-HSL (m/z : 228) was loaded as the reference (top chromatogram). C8-HSL was detected as the only signaling molecule produced by *P. pnomenus* DSM 16536^T (2nd chromatogram from top), *P. sputorum* DSM 21091^T (3rd chromatogram from top), *P. oxalativorans* DSM 23570^T (4th chromatogram from top) and *P. vervacti* DSM 23571^T (bottom chromatogram).



Supplementary Figure 2. Comparison of the 20bp *lux* box sequences located upstream of *cepI*, *soli*, *rhlI* and *luxI* of *Pandora* species. **(Figure 1a)** Sequences used for comparison are *cepI* K56-2, *Burkholderia cenocepacia* K56-2 (Lewenza et al., 1999); *soli* AW1, *Ralstonia solanacearum* AW1 (Flavier et al., 1997); *rhlI* PAO1, *Pseudomonas aeruginosa* PAO1 (Latifi et al., 1995). Black arrows indicate inverted repeats of the palindromic sequences and shaded boxes indicate identical nucleotide against the consensus sequence (Gray et al., 1994). Consensus sequence abbreviations are: N is A, T, C, or G; R is A or G; S is C or G; Y is T or C; and X is N or a gap in the sequence. **(Figure 1b)** Graphical representation of the *lux* box sequences.



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