SUPPLEMENTARY DATA

Necessity requires no decision: carbon source-dependent pattern of antimicrobial activity and gene expression in *Pseudomonas donghuensis* P482

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Supplementary Figures

Figure S1. Antibiosis assay for P482 wt and its mutants against *Dickeya solani* IFB102 strain on the minimal M9 medium with either 0.4% glucose or 0.4% glycerol. The photographs show representative images of the inhibition zones observed in one biological replicate (each column shows results from a single plate). The inhibition zone obtained for P482 wt served as a reference against which the diameters of the inhibition zones produced by the mutants were compared.

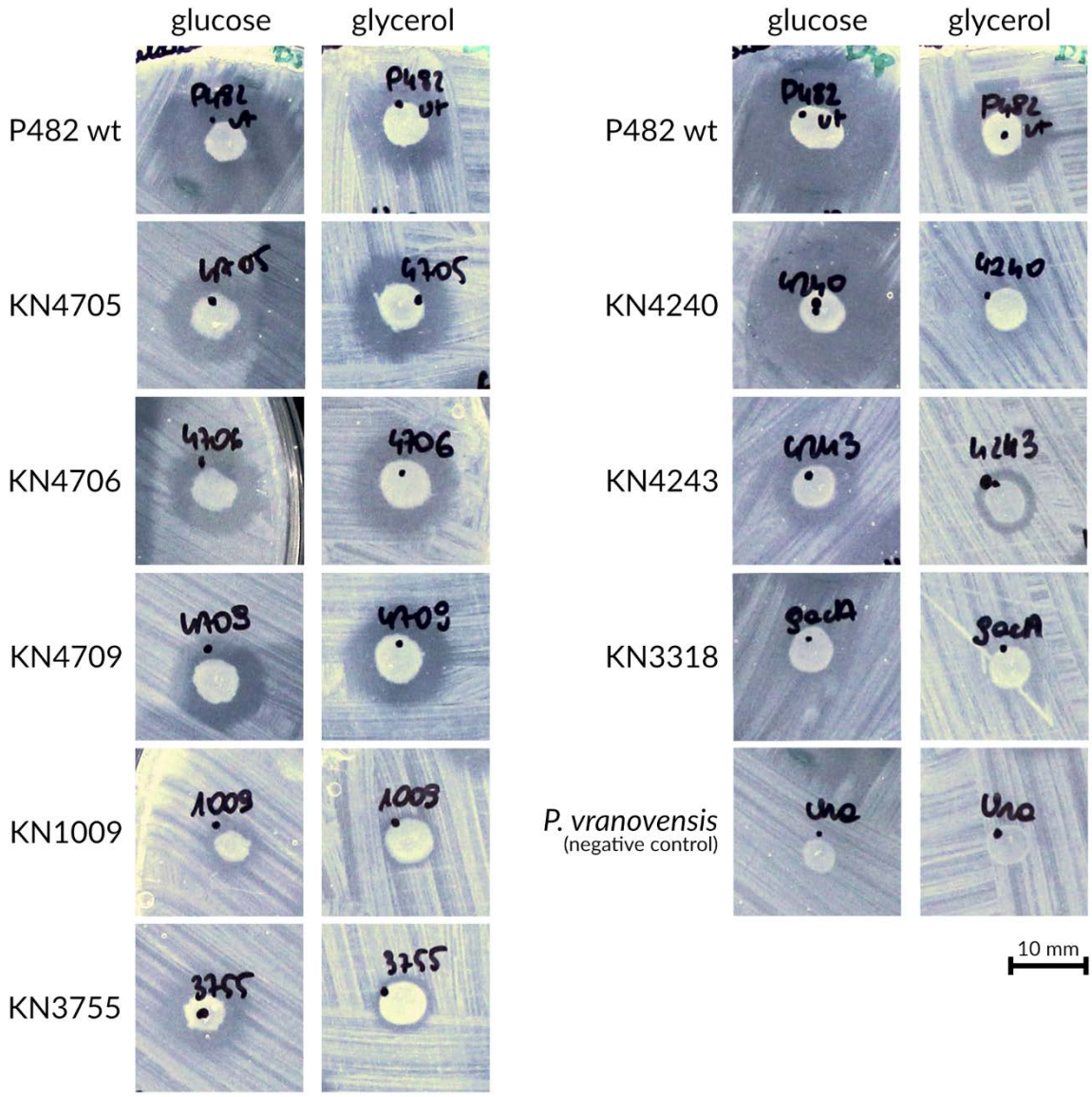


Figure S2. Graphic representation of ORF organization in the P482 gene "cluster 17". Grey arrows represent identified promoter sequences. Asterisks mark genes targeted in pKNOCK site-directed mutagenesis. The letters above the ORFs represent functional category of the product of each gene as in COG (Clusters of Orthologous Genes) classification assigned by the eggNOG-mapper tool (Huerta-Cepas *et al.*, 2017): Q – secondary metabolites biosynthesis, transport and catabolism, H – coenzyme transport and metabolism, I – lipid transport and metabolism, S – function unknown, V – defense mechanisms, E – amino acid transport and metabolism, G – carbohydrate transport and metabolism, P – inorganic ion transport and metabolism, M – cell wall/membrane/envelope biogenesis, U – intracellular trafficking, secretion, and vesicular transport. Information concerning gene annotation can be found in Table 1.

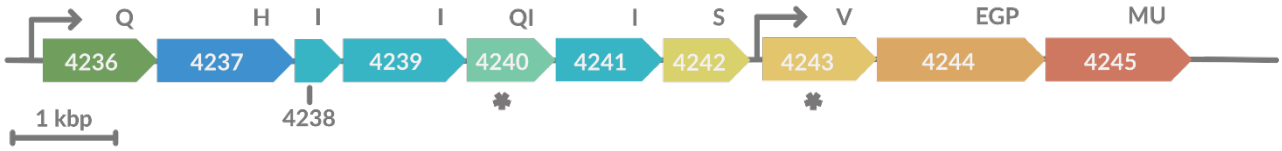


Figure S3. Growth inhibition of *Dickeya solani* IFB0102 and *Pseudomonas syringae* pv. *syringae* Pss762 by *Pseudomonas donghuensis* P482 wt and its mutants on LB-agar medium. The test was conducted according to the antibiosis assay on the M9 medium described in the Materials and Methods section. The bars represent the percentage of the growth inhibition zone obtained for the P482 wt strain under given condition. Error bars represent standard deviation.

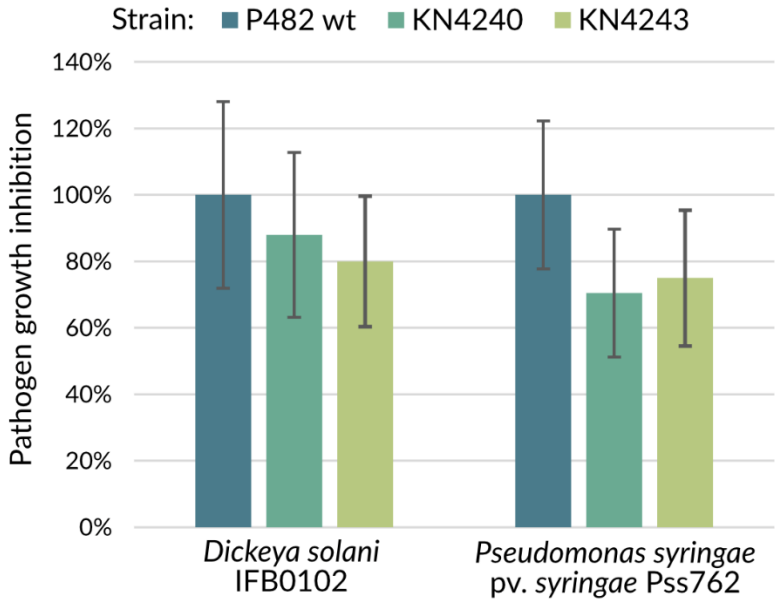


Figure S4. Results of analyses of the stability of r**eference genes as calculated by RefFinder with four algorithms: (a) ΔCT, (b) NormFinder, (c) BestKeeper, (d) geNorm**. The lowest values obtained with each of the algorithms indicate the most stable reference genes. The overall ranking based on geometric mean of the gene ranks from each of the algorithms can be found in Figure 2b.

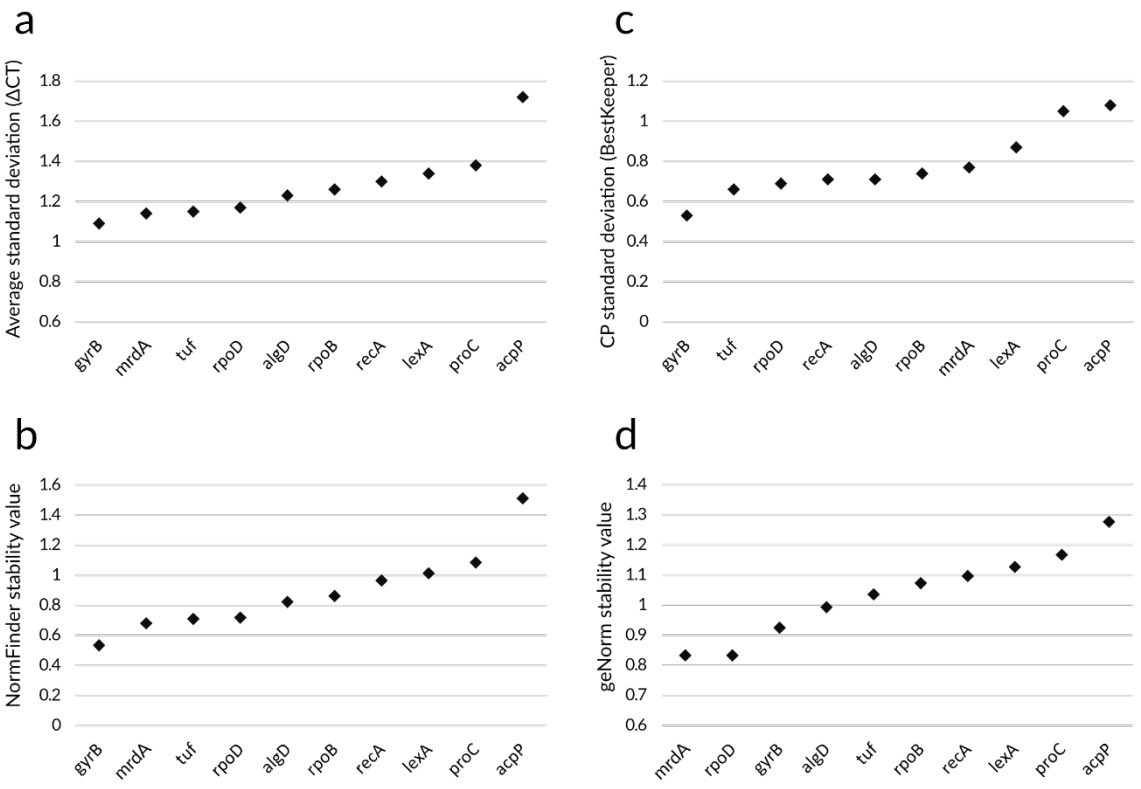


Figure S5. Growth curves for *Pseudomonas donghuensis* P482 wild type strain and its mutants were determined prior to all performed tests. The presented growth curves were used to select the endpoints for cultures used in gene expression experiments. To obtain the growth curves the strains were pre-cultured for 24 h at 28°C in the same type of medium that was later used in the growth curve assay. The optical density of precultures was measured and adjusted so that 10 µl of a preculture (1 McF) were added to 200 µl of a fresh M9 medium supplemented with (a) 0.4% glucose or (b) 0.4% glycerol. The 96-well plate with test cultures was placed in the chamber of Epoch™ Microplate Spectrophotometer (BioTek, USA). The protocol comprised 44 h of culture (with shaking) at 28°C with 600 nm absorbance measurements (OD600) every 20 minutes; 4 replicates of each sample were measured. Mean value for each readout was used to produce growth curves of each strain under given conditions.

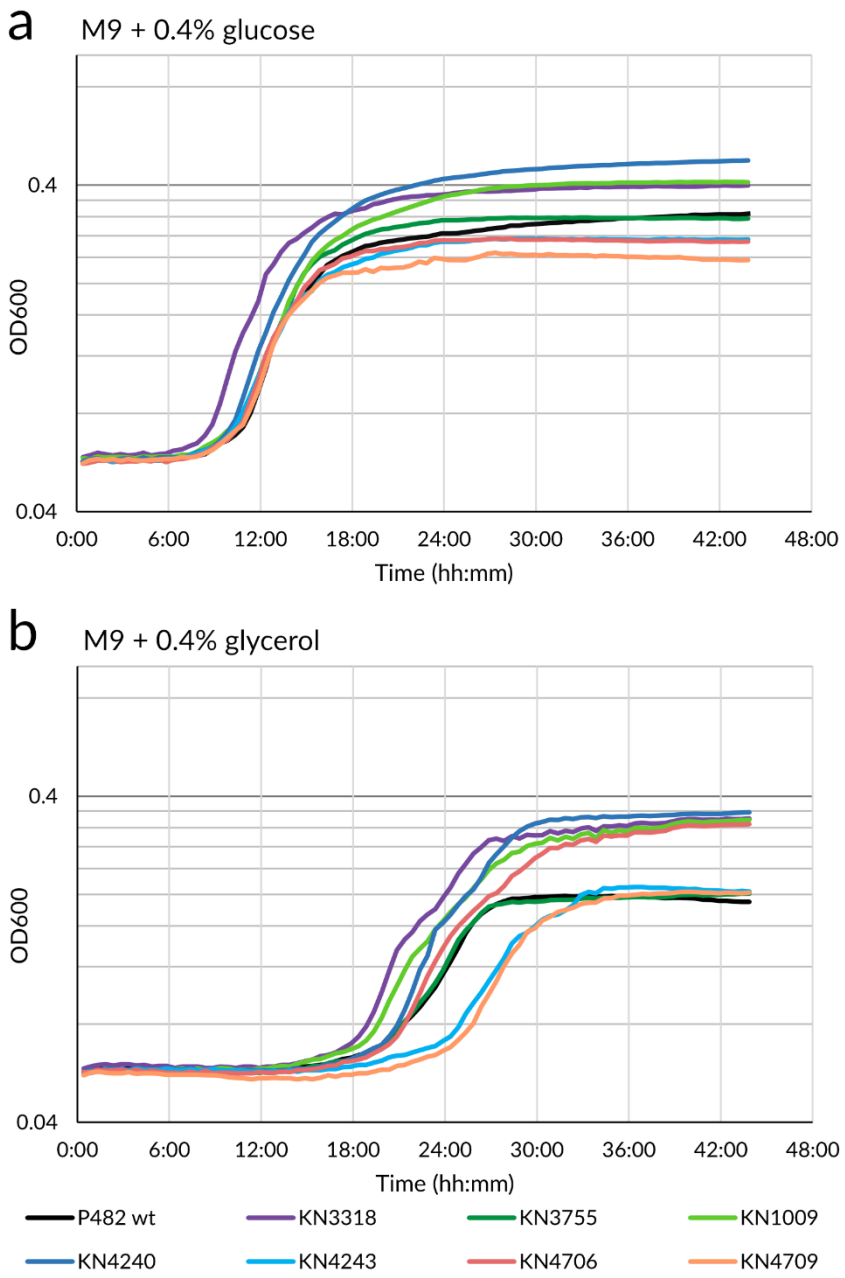
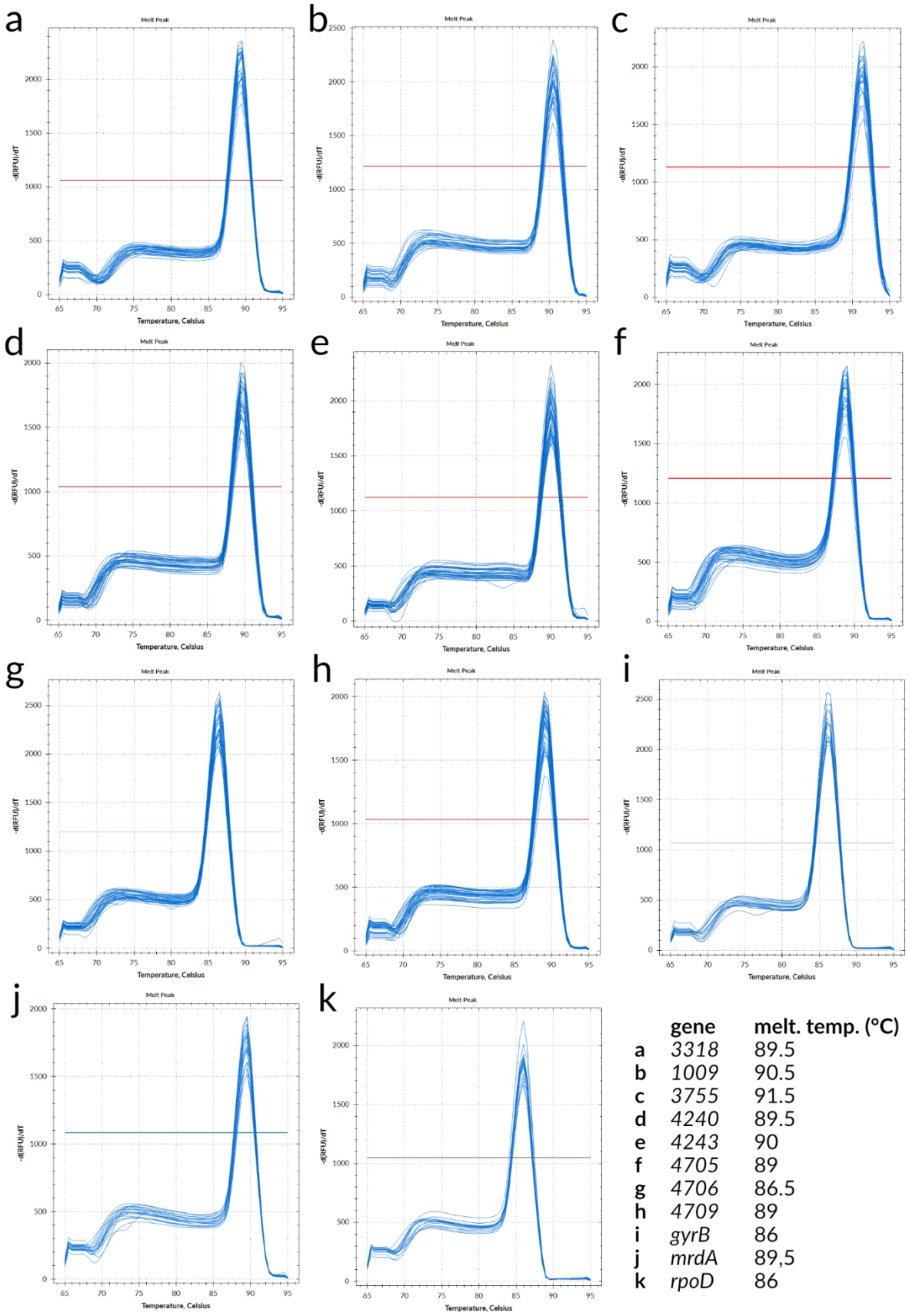
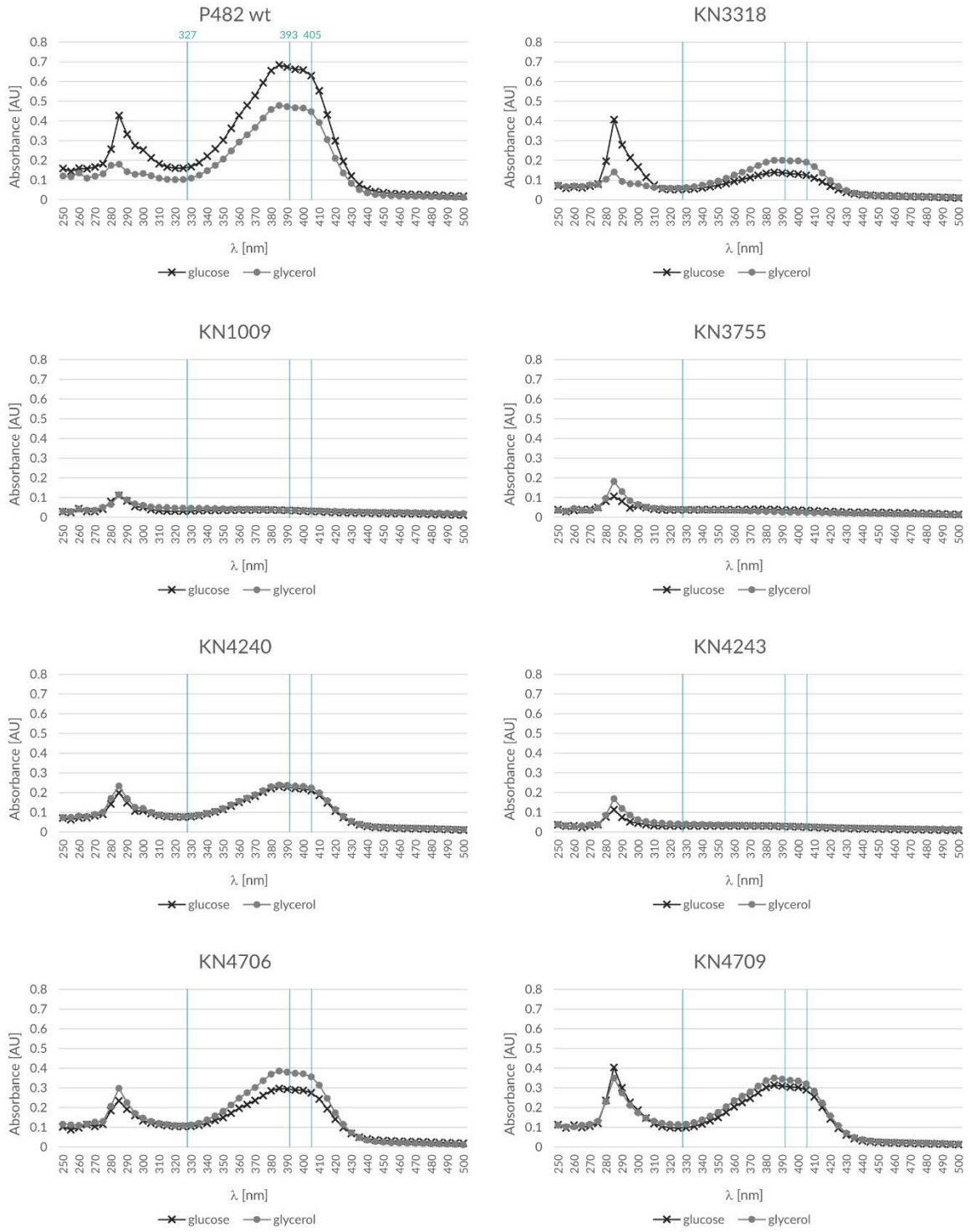


Figure S6. Melting curves of qPCR P482 targets and reference genes. All qPCR reactions in the study were followed by a melt curve step (65-95°C, increment: 0.5°C/5 sec). Each plot is a combination of peaks obtained for one target in at least 10 reactions using different P482 cDNA samples as matrix.



	gene	melt. temp. (°C)
a	*3318*	89.5
b	*1009*	90.5
c	*3755*	91.5
d	*4240*	89.5
e	*4243*	90
f	*4705*	89
g	*4706*	86.5
h	*4709*	89
i	*gyrB*	86
j	*mrdA*	89,5
k	*rpoD*	86

Figure S7. UV-visible absorption spectra of the culture supernatants of P482 wt and its mutants. To obtain the spectra, P482 wt and its mutants were cultured in liquid M9-glucose or M9-glycerol medium for 96 hours. Supernatants were collected after the centrifugation of cultures and their UV-Vis absorbance spectra were measured in the range of 200-550 nm (Epoch™ Microplate Spectrophotometer, BioTek, USA). The presence of produced iron chelators (7-HT and pyoverdine) in each supernatant was determined by the observation of characteristic absorption peaks: one peak at approximately 405 nm for pyoverdine and two peaks at approximately 330 and 392 nm for 7-HT (Jiang *et al.*, 2016).



Supplementary Tables

Table S1. Primers designed and used in the study.

Target gene/sequence (GenBank locus tag)	**Encoded protein/Description**	**Primers**	**Primer sequences [1]**	**Amplicon length (bp)**
RT-qPCR reference gene selection primers				
rpoB (BV82_1963)	RpoB, the β subunit of bacterial RNA polymerase	P482_rpoB_F P482_rpoB_R	5' TTCACCACGATCCACATCCA 5' CTTCAGCACCCACATAAACGA	147
rpoD (BV82_1895)	RpoD (σ^{70}), a "housekeeping" sigma factor involved in initiation of transcription	P482_rpoD _F P482_rpoD_R	5' CCACGACGGTATTCGAACTT 5' CGTGCCAAGAAAGAAATGGT	152
gyrB (BV82_2296)	DNA gyrase subunit B	P482_gyrB_F P482_gyrB_R	5' ATCGACAAGCTGCGCTATCA 5' CGGCTGAGCGATGTAGATGT	144
mrdA/pbp-2 (BV82_4935)	Penicillin binding protein 2	P482_mrdA_F P482_mrdA_R	5' CTTGATCGCTACCACCTGAG 5'vCAAGTCGGACTGGAACAAGG	138
recA (BV82_0583)	Recombinase A	P482_recA_F P482_recA_R	5' CTACCTGTGCCTTCGTTGAC 5' CAGCATGTCGGTGATTTCCA	133
lexA (BV82_3405)	Transcriptional repressor LexA	P482_lexA_F P482_lexA_R	5' CGTTCAAAGCGCTTGATGGT 5' CCGACTACCTGCTCAAGGTG	153
tuf (BV82_0583)	Elongation factor Tu	P482_tuf_F P482_tuf_R	5' CCCTACATCGTGGTCTTCCT 5' GAACCAATGATGATCGGAGTG	136
algD (BV82_3321)	GDP-mannose 6-dehydrogenase	P482_algD_F P482_algD_R	5' GAATATCAGCATCTTTGGATTGGG 5' CTGGTTGATCATGTCGATCTTGG	120
proC (BV82_2548)	Pyrroline-5-carboxylate reductase	P482_proC_F P482_proC_R	5' CAATCGAGACCACCAGTTGC 5' GCATCGAGATGTTCGCAGAC	140
acpP (BV82_4055)[2]	Acyl carrier protein	P482_acpP_F P482_acpP_R	5' GTCGATAGCAGCTTGAACAG 5' AGAAGTGAAGAACGAATCTTCC	150
Primers for RT-qPCR targets of interest				
BV82_1009	A putative NRPS for chromophore synthesis (Krzyżanowska *et al*., 2016)	P482_1009_F P482_1009_R	5' CAATGGCAGAGCGAATAC 5' CCGTCCTCAACCAGTAAG	140

BV82_3755	Partially similar to the PdvD pyoverdine synthase of *P.aeruginosa* (Krzyżanowska *et al.*, 2016)	P482_3755_F P482_3755_R	5’ AAAGCGAACGACATGAACAG 5’ CTGGCGTACCTGATCTACAC	151
BV82_4705	Bacterial regulatory s, tetR family protein (7-HT biosynthesis cluster)	P482_4705_F P482_4705_R	5’ CTTCCTCAGCAACGAATCCA 5’ GACCATCGAGGTGATCATCTG	150
BV82_4706	HpcH/HpaI aldolase/citrate lyase family protein (7-HT biosynthesis cluster)	P482_4706_F P482_4706_R	5’ AGGGCAGTTTCGATATTCGG 5’ GTCATGATGTCCAAGGTCGAG	127
BV82_4709	Acyl-CoA dehydrogenase, C-terminal domain protein	P482_4709_F P482_4709_R	5’ GATTTCATGCTGATCGTGACC 5’ AGGTTGTCGAAATACAGCGG	157
BV82_4240	SDR family NAD(P)-dependent oxidoreductase	P482_4240_F P482_4240_R	5’ AACCTGATGAACAAGACCGTG 5’ TAGAAGGCATCCAAGTGCAG	145
BV82_4243	Efflux transporter, RND family, MFP subunit	P482_4243_F P482_4243_R	5’ CGCCAATTTCAAGGAAACCC 5’ CTACCTTGGTGAAGTTGCCG	178
gacA (BV82_3318)	GacA, a response regulator of the two-component GacS/GacA system	P482_3318_F P482_3318_R	5’ CAATTTGCGGGCTGATGTAG 5’ GGTGACCGTCTGTGAAGAAG	148
***Primers used in site-directed mutagenesis*[3]**				
BV82_4240	BV82_4240 gene fragment for cloning into pKNOCK vector	F_XbaI_KN4240 R_XhoI_KN4240	5’ ATTATTCTAGAATTGGATGCCTTCTACCTGCT 5’ AATTTCTCGAGACGTCTGAGTAGGCGCTCAT	394
BV82_4243	BV82_4243 gene fragment for cloning into pKNOCK vector	F_XbaI_KN4243 R_XhoI_KN4243	5’ ATTATTCTAGAAACAGGGCGAACTGCTCTAC 5’ AATTTCTCGAGGCGGTATTCCTCGACTTTCA	392
pKNOCK vector[4]	pKNOCK-Km backbone	F_pKNOCK_backbone[5] R_pKNOCK_backbone[5]	5’ GGTGCCCTGAATGAACTCCA 5’ AAAAGCGGCCATTTTCCAC	-
pKNOCK vector[4]	pKNOCK insert flanking region	F_outof_pKNOCK[5] R_outof_pKNOCK	5’ CACGTACTAAGCTCTCATGTTTGAACA 5’ CTGGCAATTCCGGTTCGCT	-

[1] Synthesis of oligonucleotides was outsourced to Sigma-Aldrich (USA).
[2] *acpP* gene was excluded from qbase+ geNorm analysis.[3] Sequences recognised by restriction enzymes (XbaI and XhoI) are underlined
[4] Alexeyev, 1999
[5] Krzyżanowska *et al.*, 2016

Table S2. Ranking of the RefFinder comprehensive reference gene stability. The comprehensive stability value (CSV) in the RefFinder is calculated as a geometric mean of 4 ranking values for each gene obtained in each of the 4 algorithms available in RefFinder: delta CT, BestKeeper, NormFinder and geNorm (results of each calculation are presented in Figure S5). Underlined are the names of the genes selected as reference genes in this RT-qPCR study.

Stability ranking	1	2	3	4	5	6	7	8	9	10
Gene name	*gyrB*	*mrdA*	*rpoD*	*tuf*	*algD*	*rpoB*	*recA*	*lexA*	*proC*	*acpP*
RefFinder CSV	1.32	2.3	2.63	3.08	4.73	6	6.09	8	9	10

Table S3. Primer pair PCR efficiencies and slopes calculated from standard curves for both test and reference targets.

Target	**E***	**E (SE)****	**R^2**	**Slope**	**Slope error**
BV82_1009	2.00	0.05	0.997	-3.31	0.11
BV82_3318	1.95	0.01	0.999	-3.46	0.04
BV82_3755	2.05	0.02	0.999	-3.20	0.05
BV82_4240	2.06	0.02	0.998	-3.18	0.05
BV82_4243	1.97	0.05	0.991	-3.40	0.13
BV82_4705	1.97	0.05	0.979	-3.39	0.14
BV82_4706	2.03	0.02	0.999	-3.25	0.05
BV82_4709	1.95	0.02	0.998	-3.45	0.06
gyrB	1.96	0.08	0.981	-3.41	0.21
mrdA	2.11	0.05	0.994	-3.07	0.10
rpoD	1.96	0.02	0.999	-3.42	0.05

*E – primer pair efficiency calculated as in $E = 10^{-1/slope}$ (Rasmussen, 2001)
**E(SE) - efficiency standard error

Table S4. Raw data of P482 relative gene expression under given conditions. Data presented separately for each analysed gene. CNRQ = Calibrated Normalized Relative Quantities, SE = standard error (calculated with qbase+ software).

Relative gene expression (CNRQ)														
Sample ID (conditions)	**10% TSB**						**M9 glucose**					**M9 glycerol**		
Rep*	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**
3318	nd	nd	nd	0.243	0.146	0.183	nd	0.201	nd	0.040	0.019	-0.260	-0.353	-0.370
3318 (SE)	nd	nd	nd	0.031	0.018	0.024	nd	0.283	nd	0.107	0.056	0.081	0.064	0.076
1009	0.565	0.980	0.948	nd	nd	nd	0.525	0.465	nd	0.215	nd	0.257	-0.346	-0.072
1009 (SE)	0.038	0.049	0.068	nd	nd	nd	0.040	0.079	nd	0.079	nd	0.078	0.059	0.084
3755	0.791	0.946	0.947	nd	nd	nd	nd	0.297	nd	0.517	0.138	0.225	-0.143	-0.059
3755 (SE)	0.140	0.042	0.040	nd	nd	Nd	nd	0.020	nd	0.120	0.040	0.095	0.060	0.083
4240	nd	nd	nd	0.465	0.429	0.501	nd	nd	-0.107	0.167	-0.008	-0.077	-0.002	-0.214
4240 (SE)	nd	nd	nd	0.021	0.019	0.031	nd	nd	0.101	0.075	0.033	0.077	0.073	0.078
4243	nd	nd	nd	0.713	0.579	0.644	nd	nd	-0.426	-0.195	-0.399	0.081	-0.204	-0.263
4243 (SE)	nd	nd	nd	0.031	0.040	0.041	nd	nd	0.112	0.087	0.100	0.120	0.083	0.103
4705	nd	nd	nd	1.370	0.829	0.817	nd	nd	0.385	0.257	0.425	-0.430	-0.141	-0.278
4705 (SE)	nd	nd	nd	0.049	0.038	0.067	nd	nd	0.100	0.039	0.033	0.089	0.071	0.103
4706	nd	nd	nd	1.527	1.390	1.504	nd	nd	0.684	0.521	0.701	-0.598	-0.315	-0.624
4706 (SE)	nd	nd	nd	0.024	0.022	0.031	nd	nd	0.151	0.067	0.034	0.082	0.062	0.078
4709	nd	nd	nd	1.879	1.776	1.948	nd	nd	0.712	0.568	0.934	-0.890	-0.806	-0.893
4709 (SE)	nd	nd	nd	0.036	0.040	0.049	nd	nd	0.148	0.082	0.034	0.091	0.070	0.080

* biological replicate ; nd = given biological replicate was not used in RT-qPCR for the given gene expression analysis

Table S5. Student t-test results for the comparison of expression of P482 target genes under various carbon source (glucose or glycerol).

Target	p	Fold change glucose/glycerol	95% ci low	95% ci high
BV82_4709	0.02468	39.91	15.15	105.14
BV82_4706	0.02468	16.01	6.06	42.27
BV82_3318	0.02468	2.60	1.62	4.15
BV82_4705	0.02828	4.87	2.25	10.54
BV82_1009	0.18888	2.85	0.68	11.94
BV82_4243	0.26789	0.61	0.26	1.46
BV82_3755	0.26789	1.71	0.67	4.36
BV82_4240	0.35543	1.30	0.67	2.53

References:

1. Alexeyev, M. F. The pKNOCK Series of Broad-Host-Range Mobilizable Suicide Vectors for Gene Knockout and Targeted DNA Insertion into the Chromosome of Gram-Negative Bacteria. *Biotechniques* **26**, 824–828 (1999).
2. Huerta-Cepas, J. *et al.* Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Mol. Biol. Evol.* **34**, 2115–2122 (2017).
3. Jiang, Z., Chen, M., Yu, X. & Xie, Z. 7-Hydroxytropolone produced and utilized as an iron-scavenger by Pseudomonas donghuensis. *BioMetals* **29**, 817–826 (2016).
4. Krzyżanowska, D. M. *et al.* When genome-based approach meets the 'Old but Good': Revealing genes involved in the antibacterial activity of *Pseudomonas* sp. P482 against soft rot pathogens. *Front. Microbiol.* **7**, 1–18 (2016).
5. Rasmussen, R. Quantification on the LightCycler. in *Rapid Cycle Real-Time PCR* 21–34 (Springer Berlin Heidelberg, 2001). doi:10.1007/978-3-642-59524-0_3.