Table S1. Bacterial strains, plasmids and primers used in this study. Potential indicator strains are listed in Table S2.

Strain, plasmid or primer	Origin or relevant characteristic	Source or reference
Bacteria		
Pseudomonas sp. LMG 2338	WLIP producer and white line indicator	BCCM[a]
P. putida		
RW10S2	Rice rhizosphere isolate, Sri Lanka	20
CPMG2130, CPMG2131, CPMG2132, CPMG2133, CPMG2134, CPMG2135, CPMG2136	RW10S2 mutant, *gacS*::Tn*Mod*, Km^R	This study
CMPG2173	RW10S2 mutant, *wlpR*::Tn*Mod*, Km^R	This study
CMPG2170, CMPG2171, CMPG2172	RW10S2 mutant, *wlpA*::Tn*Mod*, Km^R	This study
CMPG2165, CMPG2166, CMPG2167, CMPG2168, CMPG2169	RW10S2 mutant, *wlpB*::Tn*Mod*, Km^R	This study
CMPG2120, CMPG2121, CMPG2122, CMPG2123, CMPG2124, CMPG2125, CMPG2126, CMPG2127	RW10S2 mutant, *wlpC*::Tn*Mod*, Km^R	This study
CMPG2173 (pCMPG6125)	CMPG2173 complemented strain, containing pCMPG6125, Km^R, Tet^R	This study
CMPG2173 (pCMPG6116)	CMPG2173 complemented strain, containing pCMPG6116, Km^R, Tet^R	This study
CMPG2134 (pCMPG6113)	CMPG2134 complemented strain, containing pCMPG6113, Km^R, Tet^R	This study
CMPG2134 (pCMPG6203)	CMPG2134 complemented strain, containing pCMPG6203, Km^R, Tet^R	This study
RW10S2PMRI	RW10S2 mutant, *pmrI*::Km, Km^R	17
RW10S2PMRR	RW10S2 mutant, *pmrR*::Km, Km^R	17
P. tolaasii CH36	*Agaricus bisporus* isolate, Belgium	4
Plasmids		
pHERD26T	*Pseudomonas* expression vector (P_{BAD} *araC*), Tet^R	14
pJB3Tc20	Broad-host-range cloning vectore, Ap^R, Tet^R	2
pCMPG6113	pJB3Tc20 with *gacS* from *P. putida* RW10S1	10
pCMPG6116	pHERD26T with *xtlR* from *P. putida* BW11M1 cloned in *Xba*I/*Eco*RI sites	9
pCMPG6125	pJB3Tc20 with *wlpR* from *P. putida* RW10S2 cloned	This study

	in *Xba*I/*Eco*RI sites	
pCMPG6203	pJB3Tc20 with *gacS* from *P. putida* RW10S2 cloned in *Hin*dIII/*Xba*I sites	This study
Primers		
PGPRB-6231	5'-CCACGGCATTGATTCAGGCGCA-3'	This study
PGPRB-6232	5'-AGGGCGTGGATTCGTGGCTGTT-3'	This study
PGPRB-6233	5'-CGGCGCAACTGCCGGAATTCAT-3'	This study
PGPRB-6234	5'-GGCTGGCAAAGGCGAATTGCGA-3'	This study
PGPRB-6353	5'-TGTGCCAGCAGCCGCGGTAATA-3'	This study
PGPRB-6354	5'-TGACGACAGCCATGCAGCACCT-3'	This study
PGPRB-6551	5'-TTGCTCTAGATCAGGCACCGGCCATCCATC-3'	This study
PGPRB-6552	5'-CTCGGAATTCGACTCAAGGATGAACAGGAC-3'	This study
PGPRB-7317	5'-TTGCAAGCTTTCAGGCACTCAGGTGCGCCT-3'	This study
PGPRB-7318	5'-CTCGTCTAGACCACTCGCGACGGCCCGCAC-3'	This study
PGPRB-7319	5'-GGACACCTTGGGCAGCAACTCC-3'	This study
PGPRB-7320	5'-CAGACAGCCTTTTCCCTCGGCG-3'	This study
PGPRB-7321	5'-CCTGGCACAGGCCGATTACAGC-3'	This study
PGPRB-7322	5'-TGACCTTCAGCGAGCGCAACTG-3'	This study
PGPRB-7323	5'-TCGAAGTGCCCAACCCTGACCA-3'	This study
PGPRB-7324	5'-GTCCATGTCGCGGGTTTCCTGG-3'	This study
PGPRB-7325	5'-ATGAAGGAGAAAACTCACCGAGGC-3'	This study
PGPRB-7326	5'-GGCAATCAGGTGCGACAATCTATC-3'	This study

[a] BCCM/LMG: Belgian Co-ordinated Collection of Micro-organisms (http://bccm.belspo.be/index.php) / Laboratory of Microbiology Ghent University

Table S2. Strains used in analysis of antagonistic activity spectrum of *P. putida* RW10S2

Strain	Origin or relevant characteristic	Source or reference
α-Proteobacteria		
Azospirillum brasilense Sp245	Wheat rhizosphere isolate	5
Sphingomonas wittichii RW1	River isolate	11
β-Proteobacteria		
Bordetella avium 197N	Domesticated turkey isolate	15
Burkholderia vietnamensis LMG 10927	Rice rhizosphere isolate	BCCM[a]
Variovorax paradoxus LMG 1797	Type strain	BCCM
γ-Proteobacteria		
Aeromonas hydrophilia ATCC7966	Milk tin isolate	ATCC[b]
Citrobacter freundii ATCC8090	Type strain	ATCC
Enterobacter aerogenes ATCC13048	Type strain	ATCC
Klebsiella pneumoniae ATCC13883	Neotype strain human serotype 3	ATCC
Proteus vulgaris LMM2011		
Pseudomonas aeruginosa PA14	Clinical isolate	8
PAO1	Clinical isolate	18
P. fluorescens LMG 1794	Type strain	BCCM
P. putida KT2440	Restriction-deficient isolate of *P. putida* mt-2	13
P. savastanoi pv. *savastanoi* LMG 5484	*Olea europaea* isolate, France	BCCM
P. stutzeri LMG 2333	Type strain, spinal fluid isolate	BCCM
P. syringae pv. *tomato* DC3000	Rifampicin-resistant derivative of strain DC52	3
P. tolaasii CH36	*Agaricus bisporus* isolate, Belgium	4
P. viridiflava LMG 2352	Type strain	BCCM
Salmonella enteritidis ATCC13076		ATCC
Serratia entomophila DSM12358		DSMZ[c]
Shigella flexneri LMG 10472	Serotype 2b	BCCM
Xanthomonas sp. pv. *zinnia* LMG 8692	*Zinnia elegans* isolate, Australia	BCCM
X. albilineans LMG 494	*Saccharum officinarum* isolate, Fiji	BCCM
X. alfalfae pv. *alfalfa* LMG 497	*Medicago sativa* isolate, Sudan	BCCM
X. axonopodis pv. *manihotis* LMG 784	*Manihot esculenta* isolate, Brazil	BCCM

X. campestris pv. *campestris* LMG 582	*Brassica* sp. isolate, Belgium	BCCM
X. campestris pv. *pelargonii* 10342	*Pelargonium zonale* isolate, France	1
X. citri pv. *malvacearum* LMG 761	*Gossypium* sp. isolate, Sudan	BCCM
X. hortorum pv. *hederae* LMG 7411	*Hedera helix* isolate, USA	BCCM
X. oryzae pv. *oryzae* PXO99	*Oryzae sativa* isolates, Philippines	19
PXO112		
PXO340		
X. sacchari LMG 471	*Saccharum officinarum* isolate, Guadeloupe	BCCM
X. translucens LMG 12921	*Anthurium andreanum* isolate, USA	BCCM
X. translucens pv. *cerealis* LMG 679	*Bromus inermis* isolate, USA	BCCM
X. translucens pv. *graminis* LMG 726	*Dactylis glomerata* isolate, Switzerland	BCCM
X. translucens pv. *hordei* LMG 737	*Hordeum vulgare* isolate, India	BCCM
X. vasicola pv. *holcicola* LMG 736	*Sorghum bicolor* isolate, New Zealand	BCCM
Yersinia enterocolitica LMG 7899	Glanders-like infection isolate, France	BCCM
Actinobacteria		
Mycobacterium smegmatis DSM43756		DSMZ
Rhodococcus erythropolis N11	Soil isolate	12
Firmicutes		
Bacillus megaterium ATCC13632		ATCC
Bacillus subtilis LMG 7135	Type strain	BCCM
Lactobacillus rhamnosus LMG 6400	Type strain	BCCM
Staphylococcus aureus ATCC6358		ATCC
Fungi, Ascomycetes		
Candida albicans CAI4		7
Saccharomyces cerevisiae W303-1A		6

[a] BCCM/LMG: Belgian Co-ordinated Collection of Micro-organisms / Laboratory of Microbiology Ghent University
[b] ATCC, American Type Culture Collection
[c] DSMZ: Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures

Table S3. Phenotypic characterization of *P. putida* RW10S2 and representative mutants affected in WLIP production.

Assays	WT	*wlpA*	*wlpB*	*wlpC*	*wlpR*	*wlpR*$^{+}$	*wlpR**	*gacS*	*gacS*$^{+}$	*gacS**
Antagonism[a]	8.3 ± 0.6	ND	ND	ND	ND	8.6 ± 0.3	7.6 ± 0.3	ND	7.3 ± 0.6	3.6 ± 0.8[d]
Hemolysis[b]	8.8 ± 0.8	ND	ND	ND	ND	8.3 ± 0.6	6.6 ± 0.3	ND	7.6 ± 0.6	7.3 ± 0.6
Swarming[c]	13.0 ± 1.0	ND	ND	ND	ND	12.0 ± 1.0[e]	12.0 ± 1.0	ND	12.0 ± 1.0	12.0 ± 1.0[e]

[a] Antagonism against *X. citri* pv. *malvacearum* LMG 761
[b] Hemolysis on horse blood TSA agar plate
[c] Swarming on 0.8% TSA agar
[d] Turbid halo
[e] Pattern different from wild type
WT: RW10S2 wild type; *wlpA* through *wlpR*: mutants CMPG2170, CMPG2169, CMPG2120, CMPG2173, respectively; *wlpR*$^{+}$: mutant CMPG2173 with pCMPG6125 containing *wlpR* from *P. putida* RW10S2; *wlpR**: mutant CMPG2173 with pCMPG6116 containing *xtlR* from *P. putida* BW11M1; *gacS*: mutant CPMG2134; *gacS*$^{+}$: mutant CMPG2134 with pCMPG6203 containing *gacS* from *P. putida* RW10S2; *gacS**: mutant CMPG2134 with pCMPG6113 containing *gacS* from *P. putida* RW10S1. ND: not detected. Each value (radius in mm) is the mean ± SD of three repeats.

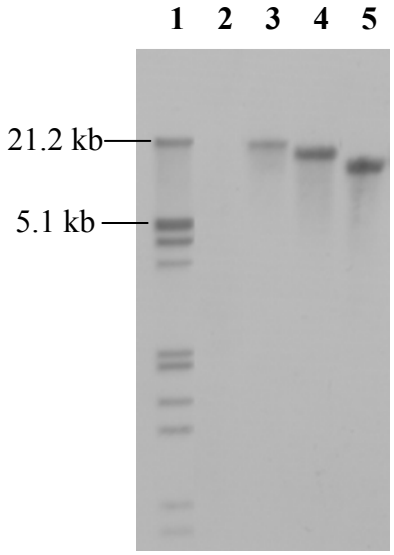


Fig. S1. Southern blot analysis of *Eco*RI-digested genomic DNA from RW10S2 wild type (2), *wlpA* (3, CMPG2170), *wlpB* (4, CMPG2169) and *wlpC* (5, CMPG2120) mutants using a probe targeting the Tn*Mod*-OKm' plasposon. DIG-labeled DNA marker III (0.1, 0.5, 0.8, 0.9, 1.3, 1.5, 1.9, 2.0, 3.5, 4.2, 4.9, 5.1, 21.2 kb) was used in lane 1.

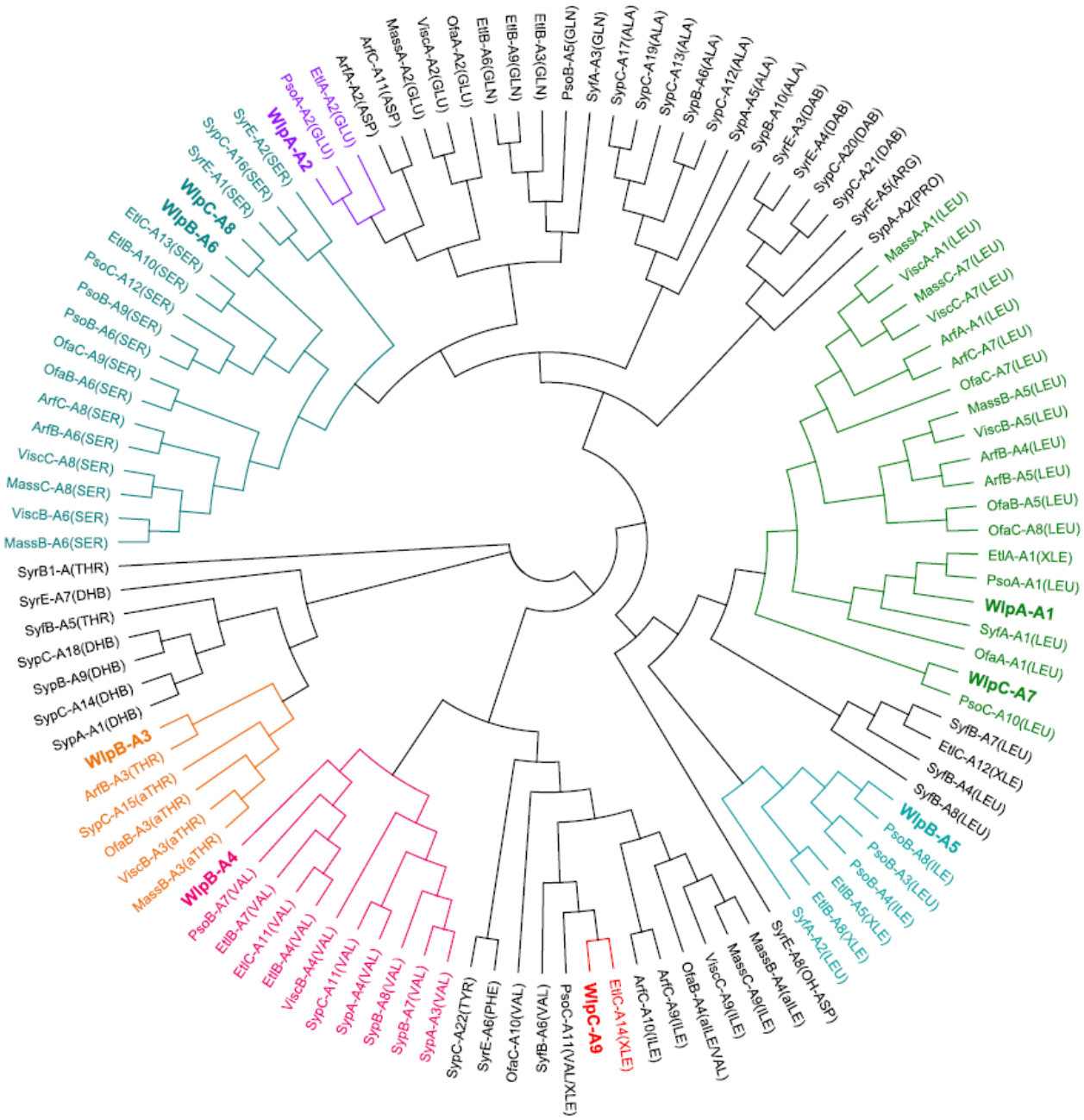


Fig. S2. Cladogram of Neighbor-Joining tree inferred from amino acid alignment of adenylation (A) domains extracted from functionally characterized *Pseudomonas* NRPSs. Lipopeptide-specific codes used for NRPS enzymes: Arf (arthrofactin, *Pseudomonas* sp. MIS38); Etl (entolysin, *P. entomophila* L48); Mass (massetolide, *P. fluorescens* SS101); Ofa (orfamide, *P. fluorescens* Pf-5); Pso (putisolvin, *P. putida* PCL1445); Syf (syringafactin, *P. syringae* DC3000); Syp (syringopeptin, *P. syringae* pv. *syringae* B301D); Syr (syringomycin, *P. syringae* pv. *syringae* strain B301D); Visc (viscosin, *P. fluorescens* SBW25); Wlp (WLIP, *P. putida* RW10S2; in bold). For each domain the amino acid substrate specificity is indicated in parentheses using the standard three-letter code. Non-protein amino acids are annotated as follows: *allo*-threonine (aTHR); 2,3-dehydro-2-aminobutyric acid (DHB); 2,4-diaminobutyric acid (DAB); 3-hydroxyaspartate (OH-ASP). Some residues that have not yet been unequivocally identified in entolysin and putisolvin II are indicated with XLE (LEU or ILE). Clusters with WLIP domains are highlighted in different colors. The tree was rooted with the divergent SyrB1 domain.

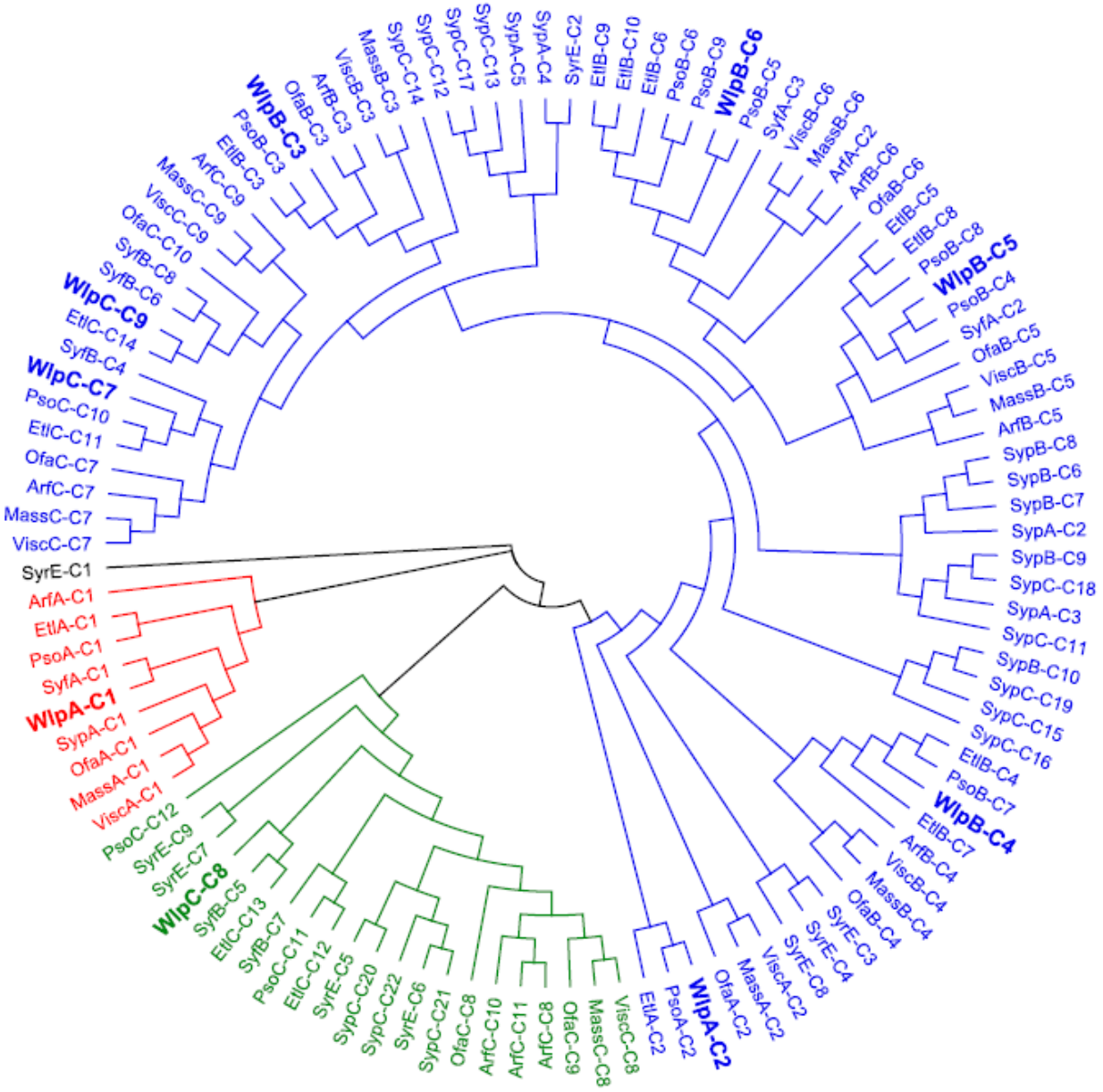
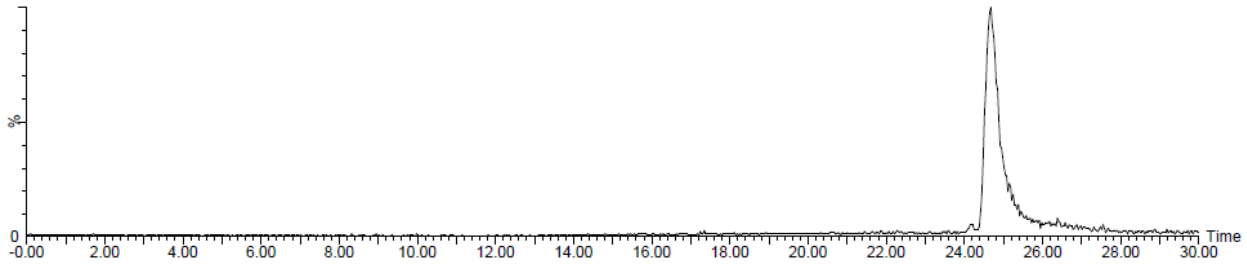
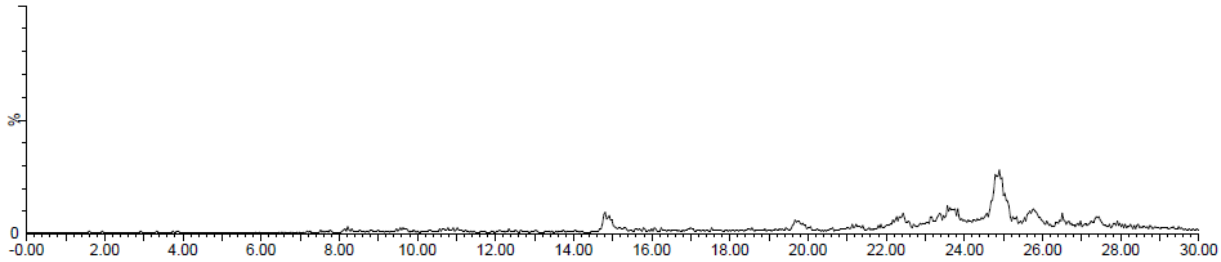


Fig. S3. Cladogram of Neighbor-Joining tree inferred from amino acid alignment of condensation (C) domains extracted from functionally characterized *Pseudomonas* NRPSs. Lipopeptide-specific codes as in supplementary figure 2. Clusters with predicted conventional domains (green), dual epimerization/condensation domains (red), and N-acylation domains (blue) are shown in different colors. The tree was rooted with the divergent C1 domain of SyrE (in black).

A



B



C

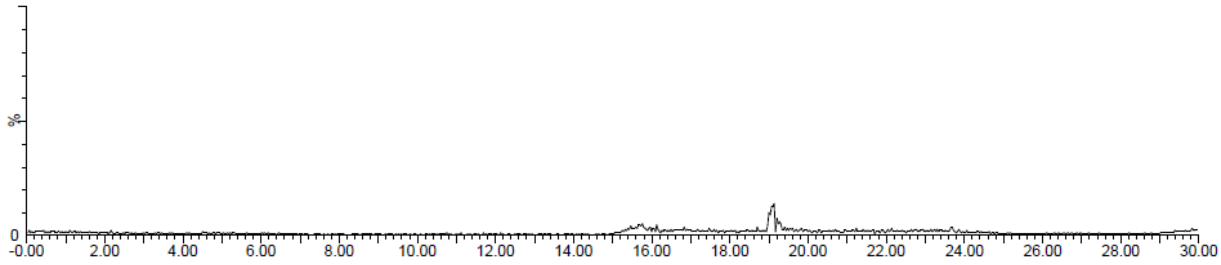


Fig. S4. HPLC chromatograms of the lipopeptide extracts from *P. putida* RW10S2 wild type (A), *gacS* mutant CMPG2134 (B), and *wlpC* mutant CMPG2120 (C). The major peak in wild-type extract, absent in the mutants, showed anti-*Xanthomonas* and hemolytic activities.

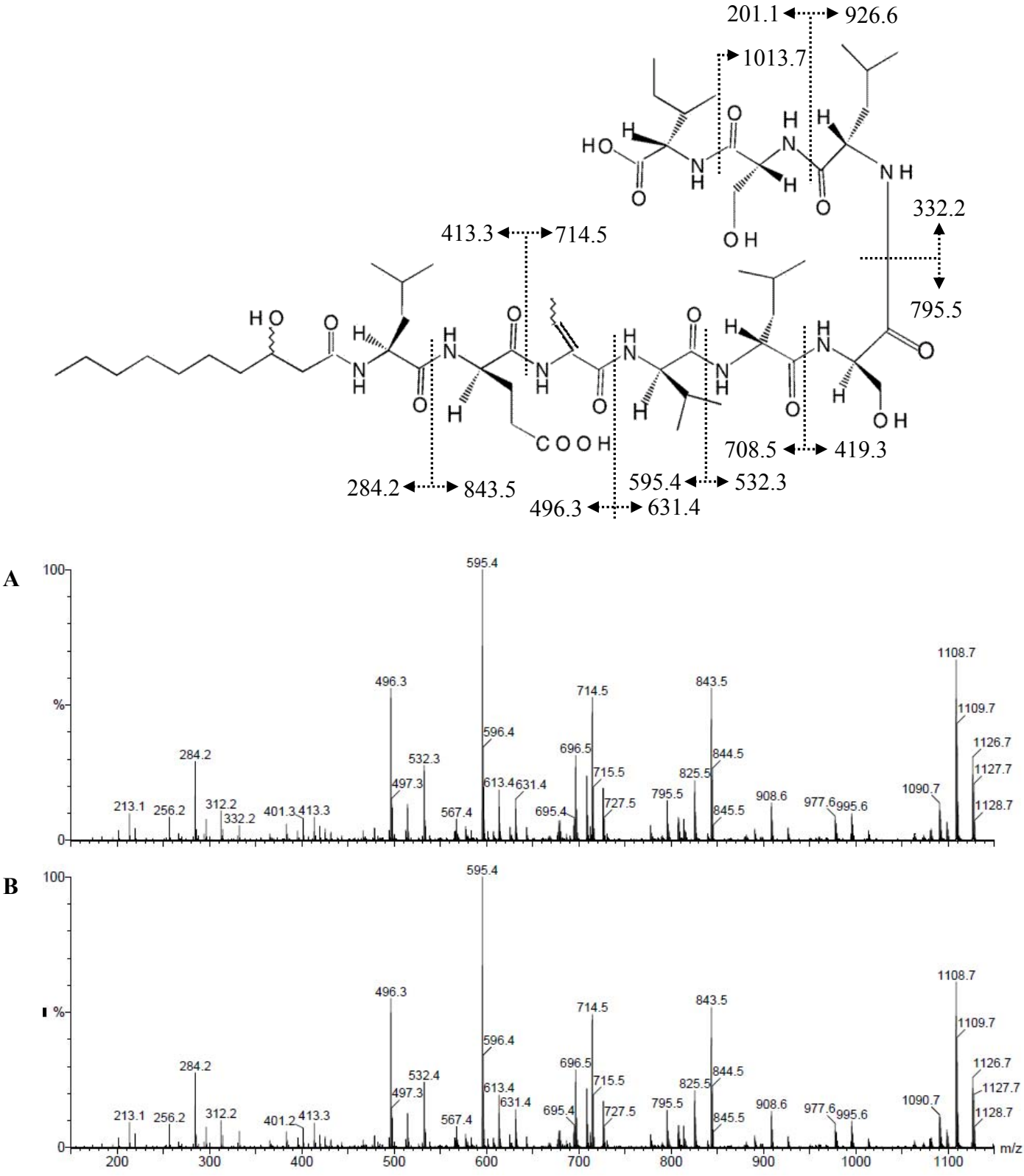


Fig. S5. MS fragmentation fingerprints of WLIP from reference WLIP producer *Pseudomonas* sp. LMG 2338 (A) and the *P. putida* RW10S2 LP (B). The ion peaks at *m*/*z* 1126.7 correspond to the protonated ion of WLIP. The other protonated ions obtained from fragmentation are indicated on the WLIP structure.

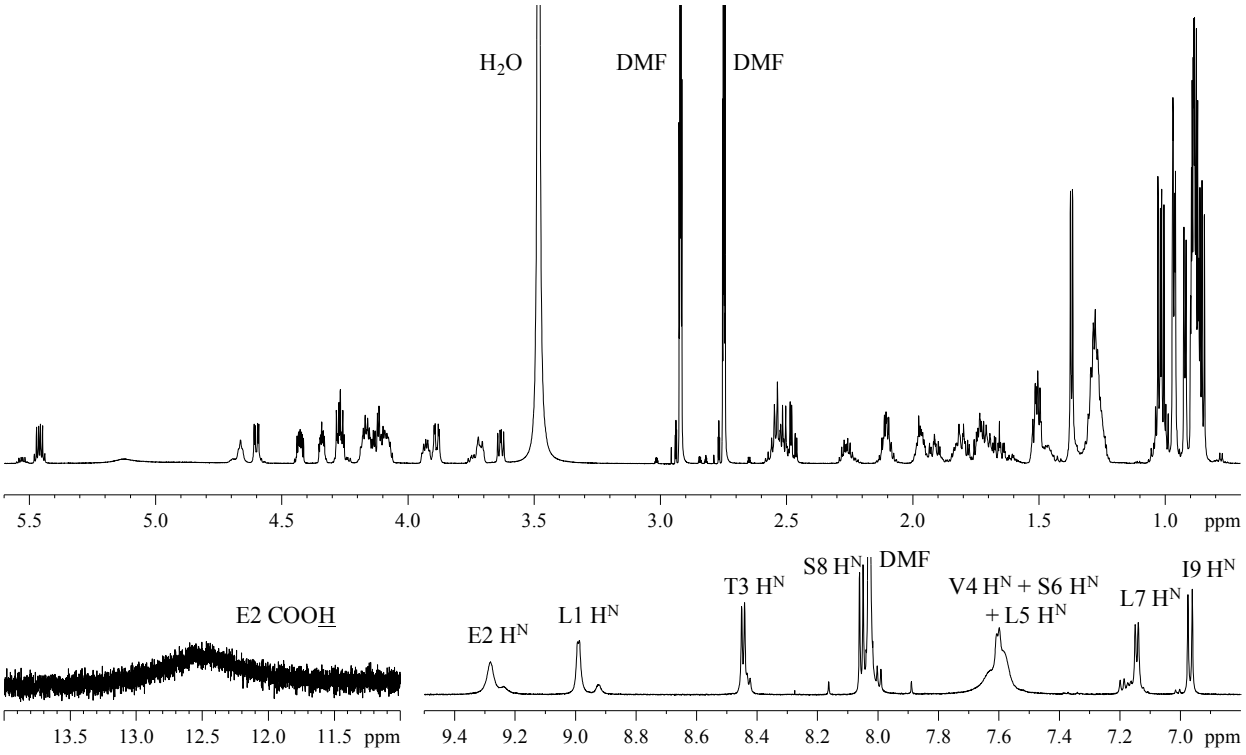


Fig. S6. 700 MHz 1D ^{1}H spectrum of WLIP in dimethylformamide (DMF-d7) solution at 25°C.

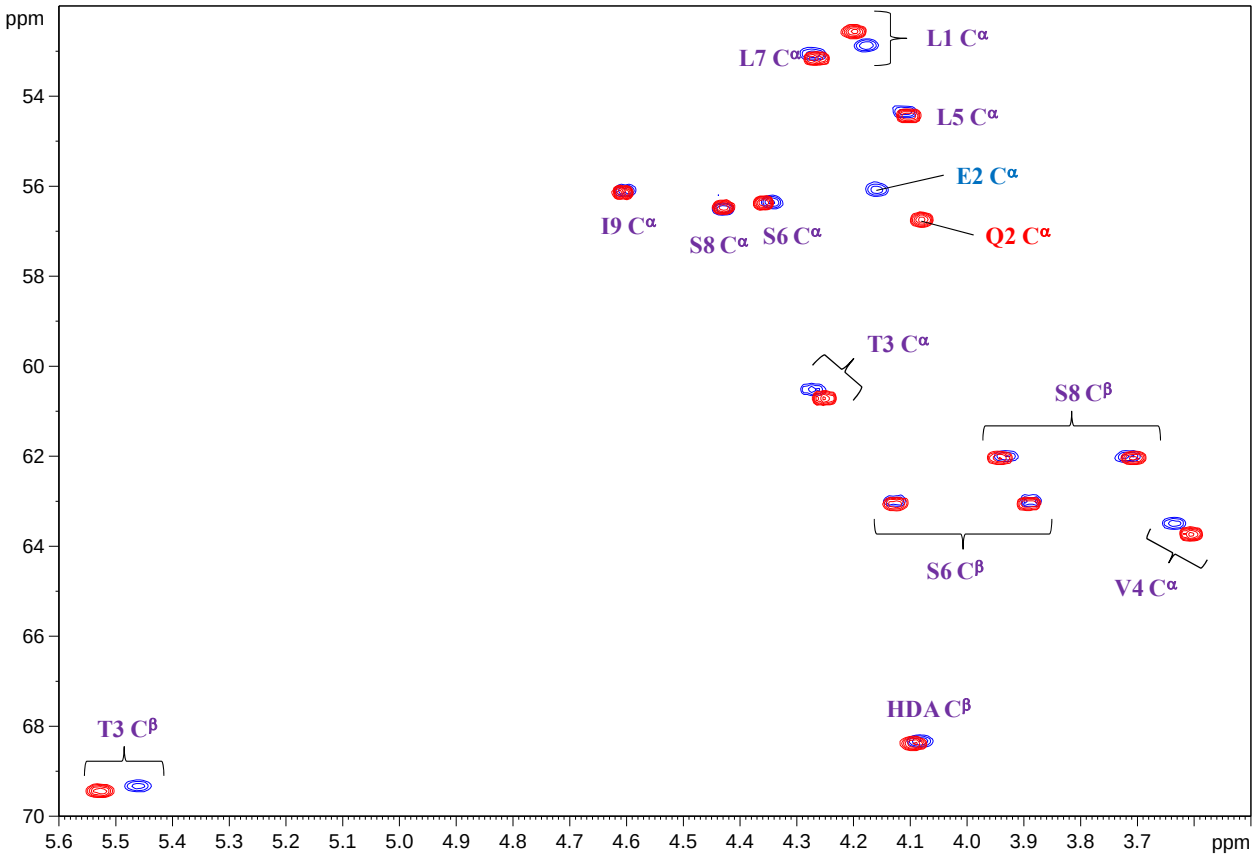


Fig. S7a. Overlay of the 700 MHz 2D ^{1}H-^{13}C HSQC spectrum of the LP of *P. putida* RW10S2 (blue) with that of pseudodesmin A (red), both measured in dimethylformamide (DMF) solution at 25°C. Only the CH$^{\alpha}$ region is shown here. Pseudodesmin A is a member of the viscosin group that differs from WLIP only by a D-glutamine instead of a D-glutamate as the second amino acid (16). All cross-peaks are labeled according to one-letter amino acid codes. No significant differences are visible in the chemical shifts between the two compounds, indicating a similar conformation and thus stereochemistry. The main difference is at the level of the Glu2→Gln2 substitution between both compounds.

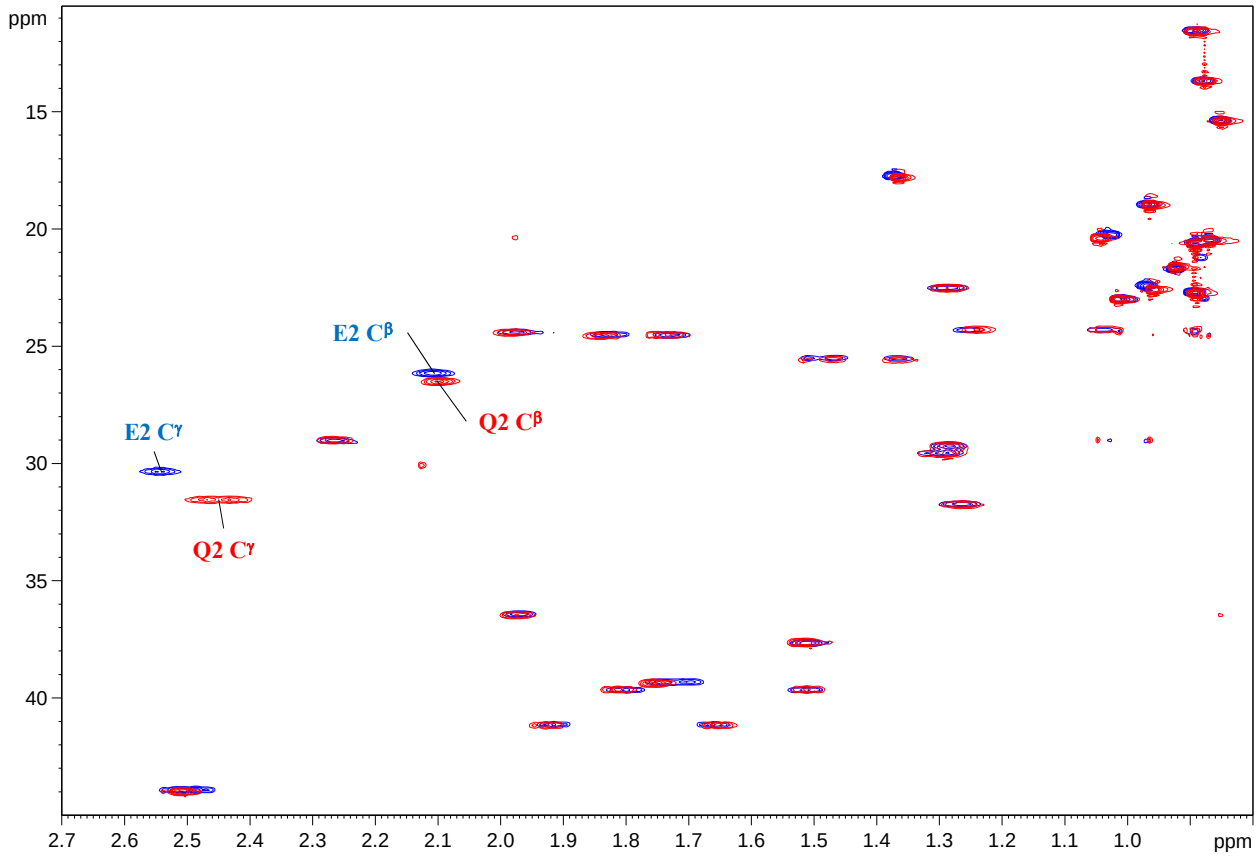


Fig. S7b. Overlay of the 700 MHz 2D ^{1}H-^{13}C HSQC spectrum of the LP of *P. putida* RW10S2 (blue) with that of pseudodesmin A (red), both measured in dimethylformamide (DMF) solution at 25°C. Only the aliphatic region is shown here. Pseudodesmin A is a member of the viscosin group that differs from WLIP only by a D-glutamine instead of a D-glutamate as the second amino acid (16). Only the cross-peaks originating from these residues are labeled on the spectrum. Other than these Glx residues, no significant differences are visible in the chemical shifts between the two compounds.

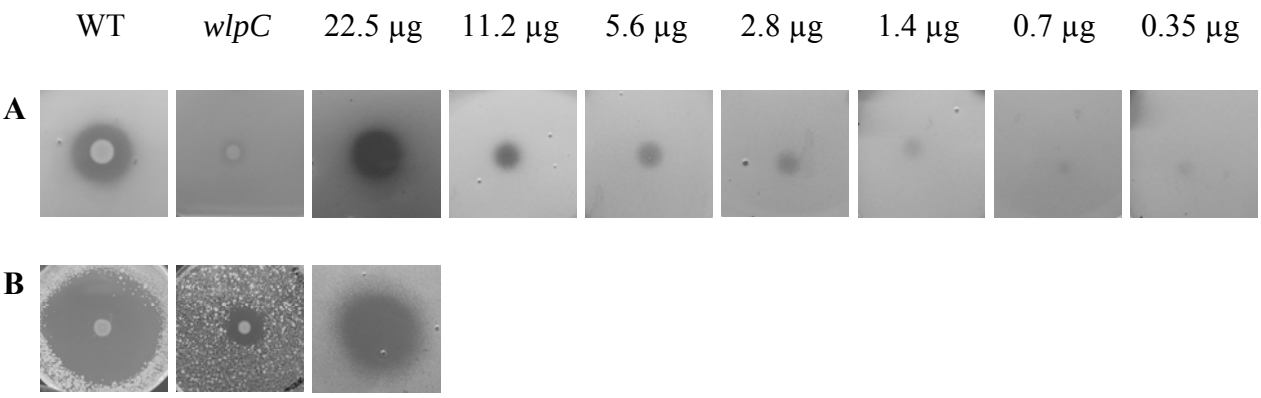


Fig. S8. Antagonistic activity of *P. putida* RW10S2 and of purified WLIP (serially diluted samples applied on plate) against (A) *X. axonopodis* pv. *manihotis* LMG 784 and (B) *B. megaterium* ATCC13632. WT: *P. putida* RW10S2 wild type; *wlpC* insertion mutant CMPG2120.
Interestingly, the WLIP-deficient *wlpA* mutant CMPG2170 produced a residual halo in a *B. megaterium* cell lawn, suggesting that some additional inhibitory substance is produced by *P. putida* RW10S2.

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