Table S1. Strains and plasmids used in this study.

	Relevant genotype, description (Genome bank number when sequenced)	References or sources
Bacillus velezensis		
GA1	Wild type (CP046386)	(1)
GA1 Δ*amyE*::cat P_{veg}-*gfpmut3* (called GA1 GFP)	GA1 disrupted of *amyE* gene; Cm^R and harbouring a constitutive transcriptional fusion (P_{veg}-*gfpmut3*).	This study
GA1 Δ*srfaA*::*cat*	GA1 deleted of *srfaA* gene; Cm^R; unable to produce surfactins	This study
S499	Wild type (NZ_CP014700)	(2)
FZB42	Wild type (NC_009725)	(3)
QST713	Wild type (NZ_CP025079)	(4)
Pseudomonas sessilinigenes		
CMR12a	Wild type (NZ_CP027706)	(5) (6)
Δ*sesA*	CMR12a disrupted of *sesA* gene; Gm^R; unable to produce sessilins	(7)
Δ*ofaBC*	CMR12a deleted of *ofaB* and *ofaC* genes; Gm^R; unable to produce orfamides	(8)
Δ*phz*	CMR12a deleted of phenazine biosynthesis operon; unable to produce phenazines	(7)
Δ*sesA-ofaBC*	CMR12a disrupted of *sesA* gene and deleted *ofaB* and *ofaC* genes; Gm^R; unable to produce sessilins and orfamides	(8)
Δ*sesA-phz*	CMR12a disrupted of *sesA* gene and deleted phenazines biosynthesis operons; Gm^R; unable to produce sessilins and phenazines	(7)
Δ*ofaAC-phz*	CMR12a deleted of *ofaB* and *ofaC* genes and phenazines biosynthesis operons; unable to produce orfamides and phenazines	(8)
Δ*sesA-ofaBC-phz*	CMR12a disrupted of *sesA* gene and deleted *ofaB* and *ofaC* genes and phenazine biosynthesis operons; Gm^R; unable to produce sessilins, orfamides and phenazines	(8)
ΔpchA	CMR12a deleted of *pchA* gene; unable to produce enantio-pyochelin	This study
ΔpvdI	CMR12a deleted of *pvdI* gene; unable to produce pyoverdine	This study
ΔpvdI-pchA	CMR12a deleted of *pvdI* and *pchA* genes; unable to produce pyoverdine and enantio-pyochelin	This study
Pseudomonas protegens		
Pf-5	Wild type; orfamide producer (NC_004129)	(9)
CHA0	*mCherry* tagged derivative of CHA0; orfamide producer (NC_021237)	(10)
Pseudomonas chlororaphis		
JV497	*mCherry* tagged derivative of JV497; non-CLPs producer (NZ_VWPC00000000)	(11)
JV395B	*mCherry* tagged derivative of JV395B; non-CLPs producer (NZ_VWPB00000000)	(11)
Pseudomonas wayambapalatensis		
RW10S2	Wild type; WLIP producer	(12)
Pseudomonas mosselii		
BW11M1	Wild type; xantholysins producer	(13)
Pseudomonas putida		
WCU_64	Wild type; putisolvins producer	(14)
Pseudomonas lactis		
SS101	Wild type; massetolides producer (NZ_CM001513)	(15)
Pseudomonas kilonensis		
F113	Wild type; viscosines producer (NC_016830)	(16)
Pseudomonas tolaasii		
CH36	Wild type; tolaasins and pseudodesmins producer	(12)
CH36Δ*tolA*	CH36 disrupted of *tolA* gene; Gm^R; unable to produce tolaasins	(12)
E. coli		
DH5α*λpir*	*supE44, ΔlacU169 (ΦlacZΔM15), recA1, endA1, hsdR17, thi-1, gyrA96, relA1, λpir*	(17)
DH5α p497	Helper strain harboring p497 plasmid	C. Keel laboratory
Plasmids		
pGEM-T	pGEM-T Easy derivative harboring the recombinant region *amyE*::*cat* P_{veg}-*gfpmut3* ; Cm^R	(18)
pOT1eM	pOT1e derivative harboring *Ptac-m-cherry* inserted in ClaI-SalI site ; Gm^R	(19)

pME6010-*eforRed*	pME6010 derivative harboring *PJ23101-eforRed* inserted in XhoI site; TetR	(T. Meyer, unpublished data)
pEMG	pSEVA212S; oriR6K, *lacZα* with two flanking I-*Sce*I sites; Km^r, Ap^r	(20)
pEMG-*pchA*	Suicide plasmid used for the deletion of *pchA*	This study
pEMG-*pvdI*	Suicide plasmid used for the deletion of *pvdI*	This study
pSW-2	oriRK2, *xylS*, P_m::*I-sceI*; Gm^R	(20)

Table S2. Primers used in this study.

	Primer Name	Primer sequence (5'->3')	Targeted genes
Deletion mutant			
***B. velezensis* GA1**			
	UpsrfaAF	TCAGCAAAACTGCGTGGTAG	*srfaA*
	UpsrfaAR	CCAATTTTCGAATTCTTTTACCGCGATAAAAAGTTATTTCCATATGTGTGC	
	DwsrfAF	CAGCTCCAGATCCTCTACGCCGGACACGCTTTATATCGTGCCGAA	
	DwsrfAR	AAGAAATGATCATAAATACC	
***P. sessilinigenes* CMR12a**			
	UppvdIF	GGCATTCTTGACCGGTCGTC	*pvdI*
	UppvdIR	GTGTTGTCCATTACACAGCCTCCATTGCATTCATCGGGAGTCATCC	
	DwpvdIF	ATGGAGGCTGTGTAATGGACAACA	
	DwpvdIR	TGTAGCGGTGTAGCAGAG	
	pvdICheckF	CCTGCTGCTGGAAGGATTGA	
	pvdICheckR	GGATCGAGCTGCCAAAGGAA	
	UppchAF	GACCAACTGCCGGCGGAT	*pchA*
	UppchAR	CCTTCAGCGATCGGCCGGTGCATCACATCTTGCGCTCCTTGCTCC	
	DwpchAF	TGATGCACCGGCCGATC	
	DwpchAR	GTGGTGAAGCTTTCCATGCC	
	pchACheckF	TCATCCACTGGAACATCGCC	
	pchACheckR	GCGGACTGATTTCCTCGGTA	
Antibiotic marker			
	CatF	CGCGGTAAAAGAATTCGAAAA	Chloramphenicol marker
	CatR	GTCCGGCGTAGAGGATCTG	
	PhleoF	GTCATAGCTGTTTCCTGCCAAAAGGGGGTTTCATTTT	Phleomycin marker
	PhleoR	ACTGGCCGTCGTTTTACTCCAATAAATGCGACACCAA	
	KanF	GTCATAGCTGTTTCCTGCTTAGCTCCTGAAAATCTCGG	Kanamycin marker
	KanR	ACTGGCCGTCGTTTTACCTGATAATTACTAATACTAGGAGAAG	
	nptIIF	GAGGATCGTTTCGCATGATT	Kanamycin marker for *Pseudomonas*
	nptIIR	CGCTCAGAAGAACTCGTCAA	
	psw-F	GGACGCTTCGCTGAAAACTA	pSW-II insertion
	psw-R	AACGTCGTGACTGGGAAAAC	

A

BSM predicted	Category	Biosynthetic gene cluster(s)
Surfactins	NRPs/CLPs	*srfaABCD*
Fengycins	NRPs/CLPs	*fenABCDE*
Iturins	NRPs/CLPs	*ituDABC*
Macrolactins	NRPs/PKs	*mlnABCDEFGHI*
Bacillaene	NRPs/PKs	*baeBCDEGHIJLMNRS*
Dihydrobacillaene	NRPs/PKs	*baeBCDEGHIJLMNRS*
Difficidin	NRPs/PKs	*dfnABDEFGHIJKLMNO*
Oxydifficidin	NRPs/PKs	*dfnABDEFGHIJKLMNO*
Bacillibactin	NRPs/ Siderophore	*dhbACEBF*
Bacilysin	Dipeptide	*bacABC*
Amylocyclicin	RiPP	*acnBACDEF*
Amylolysin	RiPP	*amlFEKRAMT*

C

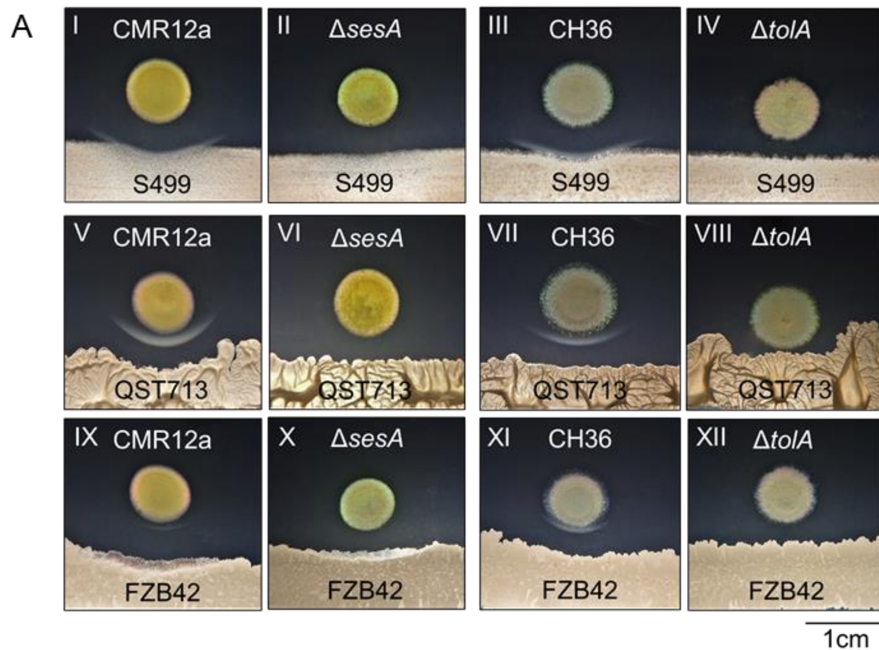
B

BSM	Main described activities	Raw formula of variants	Detected m/z $[M+H]^+$ (mass error (ppm))	EM	CAA
Surfactins[1]	Induced systemic resistance Motility	**C12** : $C_{50}H_{87}N_7O_{13}$ **C13** : $C_{51}H_{89}N_7O_{13}$ **C14** : $C_{52}H_{91}N_7O_{13}$ **C15** : $C_{53}H_{93}N_7O_{13}$ **C16** : $C_{54}H_{95}N_7O_{13}$	994.6420 (1,5) 1008.6582 (0,9) 1022.6743 (0,5) 1036.6904 (4,8) 1050.7061 (1,7)		
Iturins[2]	Antifungal	**C13** : $C_{47}H_{72}N_{12}O_{14}$ **C14** : $C_{48}H_{74}N_{12}O_{14}$ **C15** : $C_{49}H_{76}N_{12}O_{14}$ **C16** : $C_{50}H_{78}N_{12}O_{14}$ **C17** : $C_{51}H_{80}N_{12}O_{14}$	1043.5510 (1,0) 1057.5653 (2,2) 1071.5809 (2,3) 1085.5967 (2,1)		
Fengycins[3]	Antifungal	**C15** : $C_{71}H_{108}N_{12}O_{20}$ **C16** : $C_{72}H_{110}N_{12}O_{20}$ **C17** : $C_{73}H_{112}N_{12}O_{20}$ **C18** : $C_{74}H_{114}N_{12}O_{20}$ **C19** : $C_{75}H_{116}N_{12}O_{20}$	1449.7806 (4,8) 1463.8004 (1,9) 1477.8171 (2,5) 1491.8305 (2,7) 1505.8440 (4,1)		
Difficidins[4]	Antibacterial	**Difficidin** : $C_{31}H_{45}O_6P$ **Oxy-** : $C_{31}H_{45}O_7P$	567.2828 (3,2) 583.2795 (7,8)		
Bacillaenes[5]	Antibacterial	**Bacillaene** : $C_{34}H_{48}N_2O_6$ **Dihydro** : $C_{34}H_{50}N_2O_6$	581.3585 (2,3) 583.3726 (2,7)		
Macrolactins[6]	Antibacterial	**A** : $C_{24}H_{34}O_5$ (R=H) **7-O-malonyl-A** : $C_{27}H_{36}O_8$ (R=CO-CH_2-COOH) **7-O-succinyl-A** : $C_{28}H_{36}O_8$ (R=CO-CH_2-CH_2-COOH) **D** : $C_{34}H_{48}O_{13}$ (R=6-O-syccinyl-β-gluc)	435.2298 (6,7) 511.2292 (2,0) 525.2459 (0,0) 687.2991 (0,6)		
Bacillibactins[7]	Siderophore	$C_{39}H_{42}N_6O_{18}$	883.2620 (0,1)		

Figure S1. Diversity of predicted and detectable bioactive secondary metabolites (BSMs) produced by *B. velezensis* strain GA1. (A) Prediction of *B. velezensis* GA1 secondary metabolites. Biosynthetic gene clusters (genome annotation, Genbank: CP046386) were predicted by antiSMASH 6.0 (21). **(B)** GA1 BSMs and their corresponding main described activities and raw formula. Detected m/z ($[M+H]^+$) of each variant and mass error (in ppm, compared to theoretical mass) are calculated based on UPLC-MS analyses of supernatants coming from exudate mimicking (EM) and casamino acid (CAA) medium culture. Relative abundance of each BSMs is shown for both media. The numbers (superscript) next to the molecule name indicate the corresponding BSMs structure depicted in **C**. Structural changes leading to variants are indicated in red.

CLPs	Fattty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	References
Massetolide A	(3OH) C10	Leu	Glu	αThr	αIle	Leu	Ser	Leu	Ser	Ile										(22)
Pseudodesmin A	(3OH) C10	Leu	Gln	αThr	Val	Leu	Ser	Leu	Ser	Ile										(22)
WLIP	(3OH) C10	Leu	Glu	αThr	Val	Leu	Ser	Leu	Ser	Ile										(22, 23)
Orfamide B	(3OH) C14	Leu	Glu	αThr	Val	Leu	Ser	Leu	Leu	Ser	Val									(8)
Putisolvin III	C6	Leu	Glu	Leu	Leu	Gln	Ser	Val	Leu	Ser	Leu	Val	Ser							(23, 24)
Xantholysin A	(3OH) C10	Leu	Glu	Gln	Val	Leu	Gln	Ser	Val	Leu	Gln	Leu	Leu	Gln	Ile					(13, 23)
Tolaasin D	(3OH) C8	Dhb	Pro	Ser	Leu	Val	Ser	Leu	Val	Val	Gln	Leu	Val	Dhb	αThr	Leu	Hse	Dab	Lys	(25)
Sessilin A	(3OH) C8	Dhb	Pro	Ser	Leu	Val	Gln	Leu	Val	Val	Gln	Leu	Val	Dhb	αThr	Ile	Hse	Dab	Lys	(26)

Figure S2. Simplified structural representation of the cyclic lipopeptides (CLPs) produced by *Pseudomonas* strains used in this study. The fatty acid chain length is presented and the amino acid composition are depicted indicating where cyclization occurs. For each CLP, only the main form detected is represented, for more details about minor variants see the also corresponding associated references (8, 13, 22–26).


A
I CMR12a
II ΔsesA
III CH36
IV ΔtolA
S499
S499
S499
S499
V CMR12a
VI ΔsesA
VII CH36
VIII ΔtolA
QST713
QST713
QST713
QST713
IX CMR12a
X ΔsesA
XI CH36
XII ΔtolA
FZB42
FZB42
FZB42
FZB42
1cm

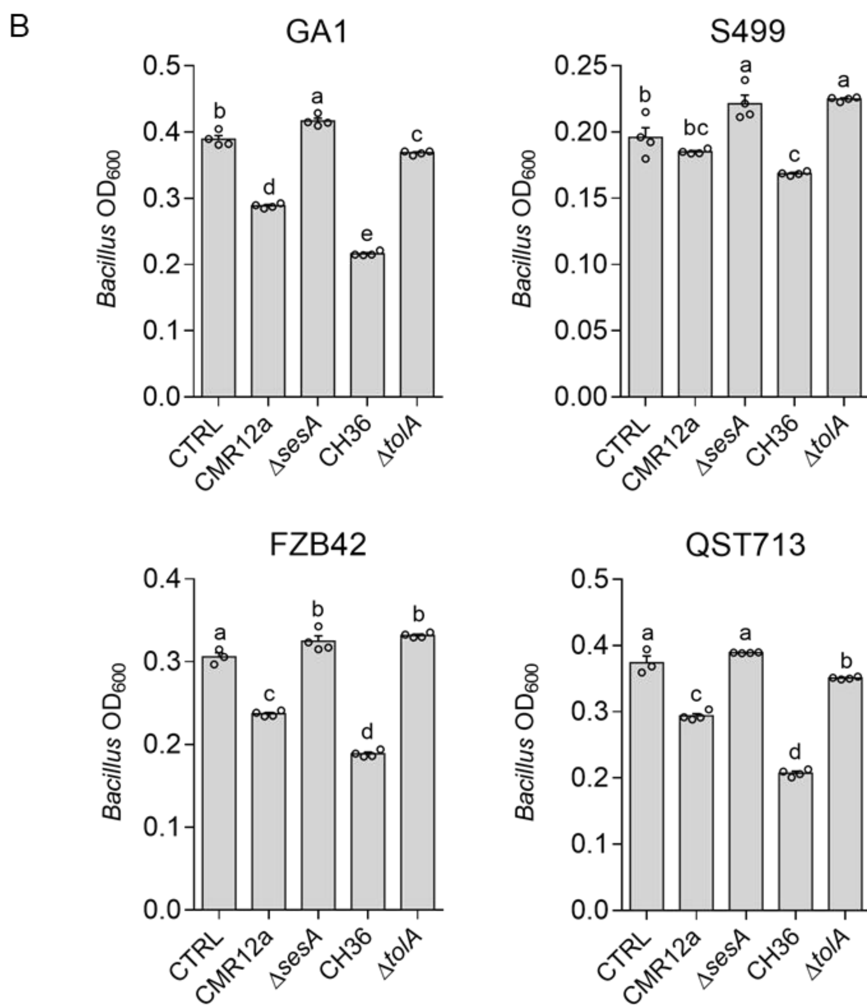

B
GA1
S499
FZB42
QST713
Bacillus OD600
CTRL
CMR12a
ΔsesA
CH36
ΔtolA

Figure S3. The interplay between surfactins and sessilins/tolaasins is conserved within *B. velezensis*. (A) The white-line formation is dependent on the co-presence of surfactins and sessilins/tolaasins producers while during the interaction between *B. velezensis* wild-type strains (S499, QST713, FZB42) and *P. sessilinigenes* CMR12a (CMR12a) or *P. tolaasii* CH36 (CH36) mutants impaired in the production of sessilins (Δ*sesA*) or tolaasins (Δ*tolA*), respectively, no white line is observed. Sessilins/tolaasins production induces slight growth inhibition of all tested *B. velezensis* strains (panels I, III, V, VII, IX, XI). No inhibition is observed in confrontation assays with *Pseudomonas* mutants unable to produce sessilins or tolaasins (panels II, IV, VI, VIII, X, XII). The figure shows one representative repetition of three biological replicates with three technical repetitions (n=9). **(B)** Optical density (at 600nm) of different *B. velezensis* (GA1, S499, FZB42 and QST713) after 10 h culture supplemented with 4% (v/v) CMR12a or CH36 supernatants and their mutants unable to produce sessilins or tolaasins, respectively. Un-supplemented culture is represented as control (CTRL). Graphs show mean ± SD calculated from four replicate cultures (n = 4). Letters a to d indicate statistically significant differences according to Tukey's HSD test (α= 0.05).

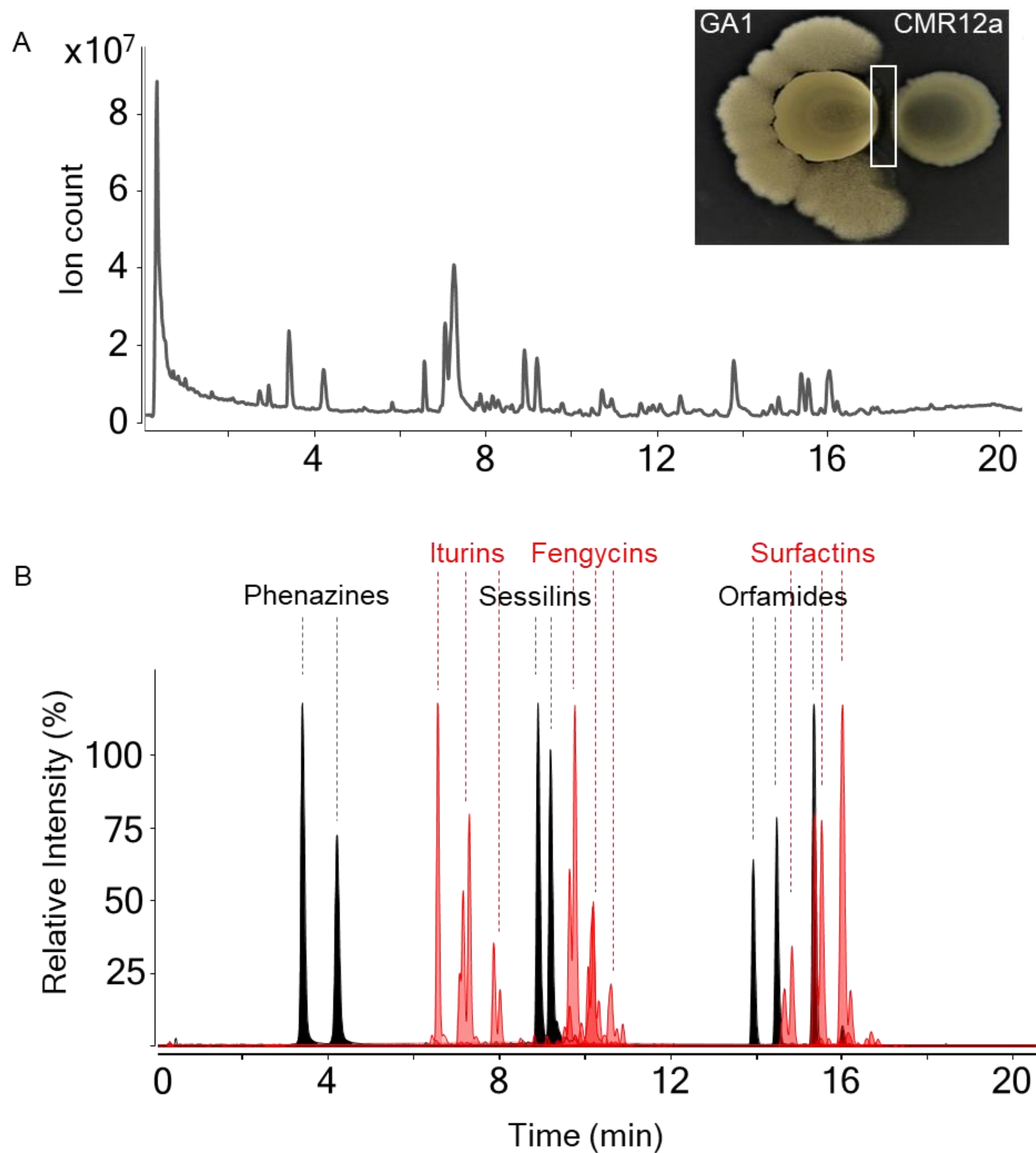


Figure S4. *P. sessilinigenes* CMR12a and *B. velezensis* GA1 BSMs production upon confrontation on solid EM medium. (A) Total ion chromatogram (TIC) and **(B)** Extracted ion chromatograms (EICs) overlaid for each BSMs produced by CMR12a (black) and GA1 (red) in the confrontation zone (labelled as a white rectangle on the upper-right confrontation figure) on solid EM medium after 24h. The intensity was fixed

to 100% for the main variant of each family. The figure shows one representative repetition of two biological replicates with three technical repetitions (n=6).

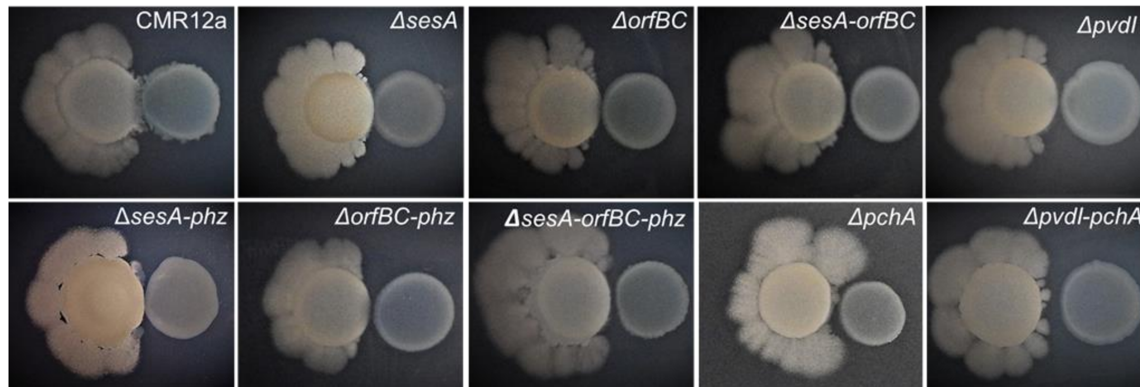


Figure S5: Effect of *P. sessilinigenes* CMR12a mutants on *B. velezensis* GA1 motility. GA1 (left colony) enhanced motility upon confrontation with CMR12a wild-type (CMR12a) or mutants repressed in the synthesis of sessilins (Δ*sesA*), orfamides (Δ*ofaBC*), orfamides and phenazines (Δ*ofaBC-phz*), sessilins and orfamides (*ΔsesA-ofaBC*), sessilins and phenazines (Δ*sesA-phz*), their triple mutant (Δ*sesA-ofaBC-phz*), pyoverdine (Δ*pvdI*), enantio-pyochelin (Δ*pchA*) or their double mutant (Δ*pvdI-pchA*) (see Table S1 for metabolome of each mutant). The figure shows one representative repetition of three biological replicates with three technical repetitions (n=9).

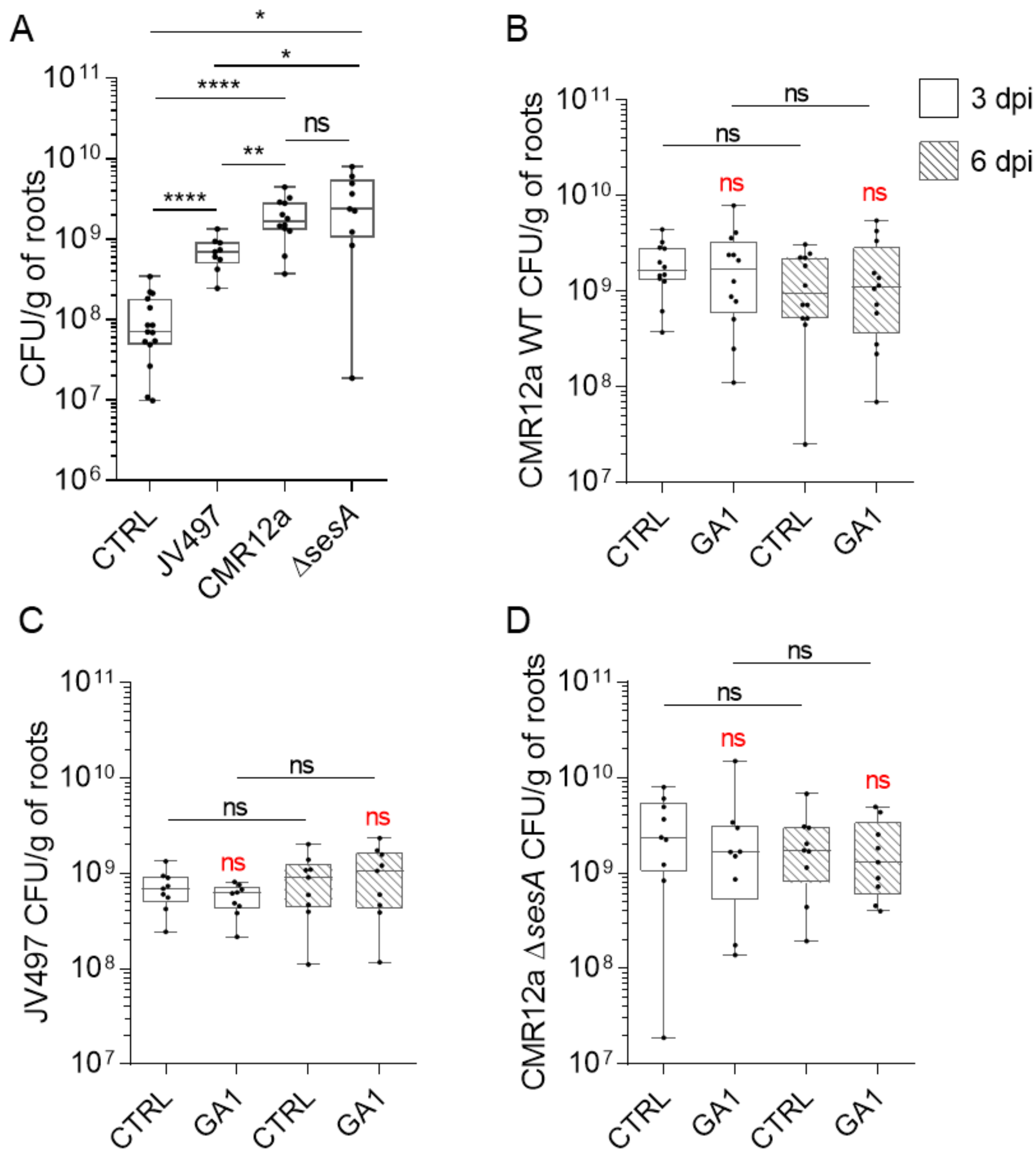


Figure S6. ***B. velezensis*** **GA1 and** ***Pseudomonas*** **spp. tomato root colonization.**

(A) GA1, JV497, CMR12a and Δ*sesA* population, expressed as colony forming units per gram of tomato roots (CFU/g of roots) recovered three days after single inoculation. Comparison between single (CTRL) and co-colonization (GA1) of tomato roots (CFU/g of roots) by CMR12a **(B),** JV497 **(C)**, and CMR12a mutant Δ*sesA* **(D)** between three (3

dpi, empty bars) and six (6 dpi, hatched bars) days after (co-)inoculation. Box plots were generated based on data from at least three independent assays each involving 6 plants per treatment (n=18). The whiskers extend to the minimum and maximum values, and the midline indicates the median. Statistical comparisons between treatments were realized with Mann–Whitney-test,''ns'' no significant difference and "****", $P<0.0001$. The statistical differences between mono- and co-inoculated conditions are indicated as red-coloured labels while the statistical differences of the same condition between 3 and 6 dpi are indicated as black-coloured labels.

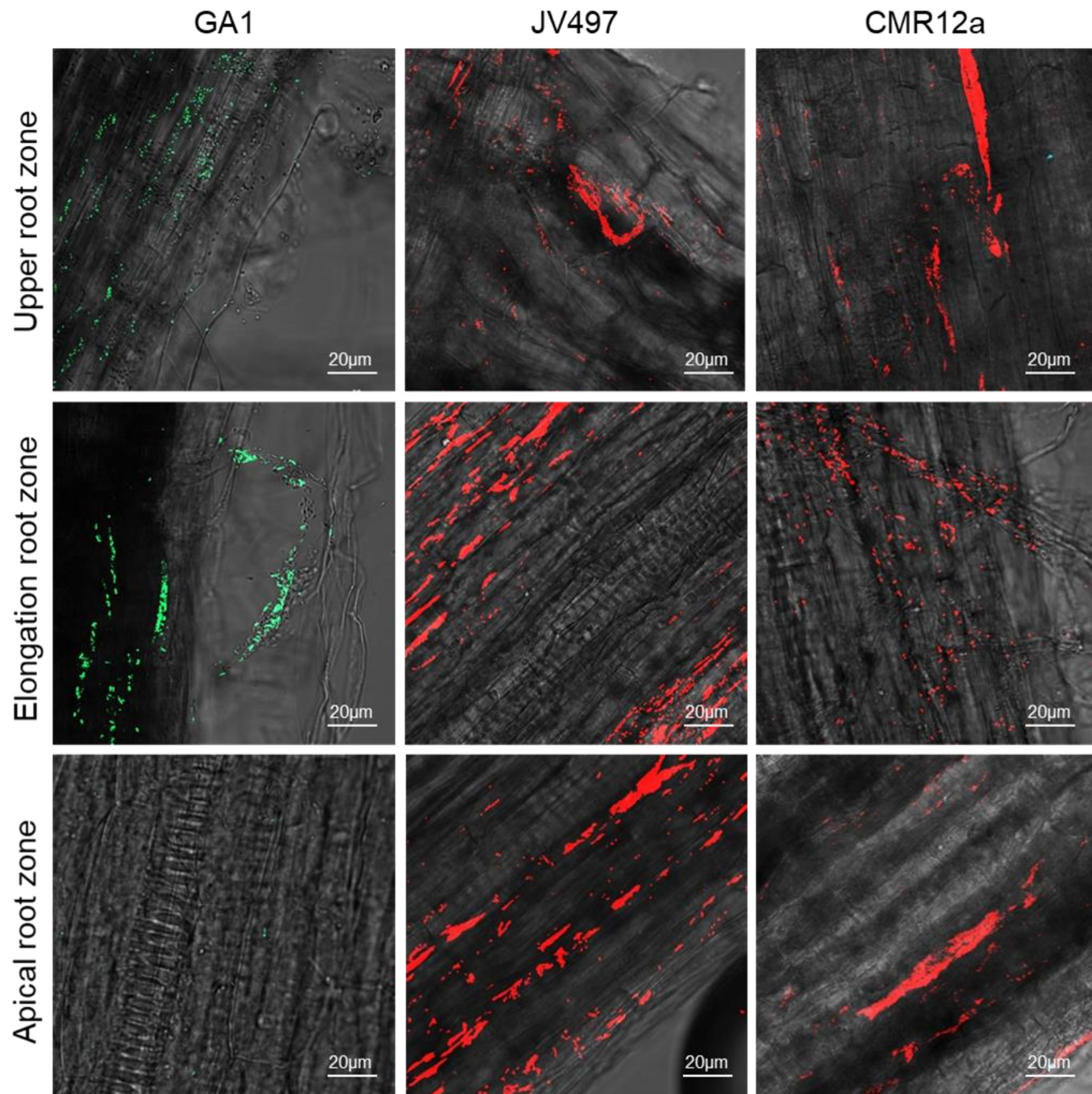


Figure S7. Confocal laser microscopy images of colonization and distribution of *B. velezensis* GA1, *P. chlororaphis* JV497 or *P. sessilinigenes* CMR12a along tomato roots. GA1, JV497, CMR12a root colonization and distribution on apical, elongation and upper root zones of tomato, 6 dpi. Cells expressing *GFPmut3* are green and correspond to GA1 cells while *mCherry*-labeled cells are red and correspond to

Pseudomonas spp. cells. The grey backgrounds correspond to root views observed with transmitted light. Images are representative of the analysis of 9 images per condition.

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