Supplementary figures

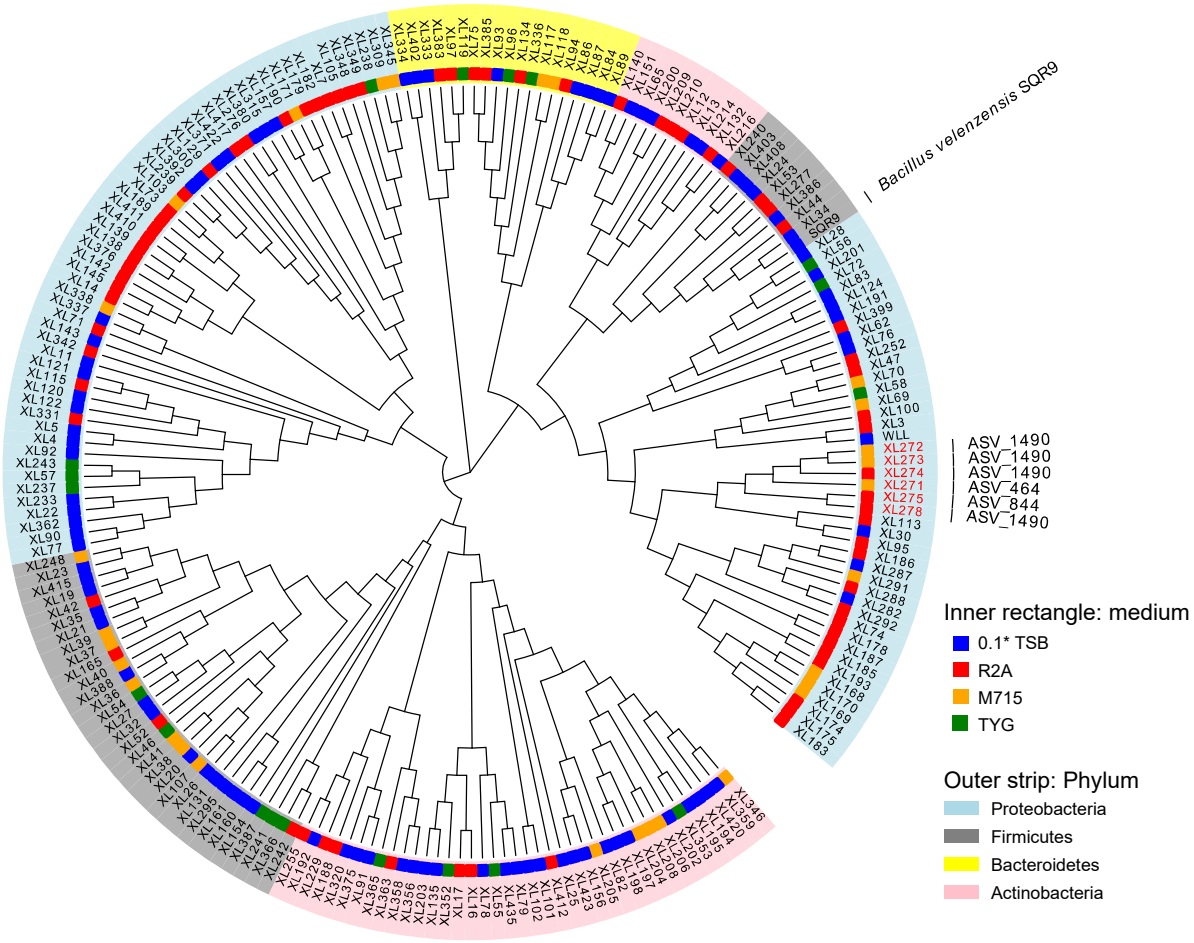

Fig. S1 Cladogram showing phylogenetic relationships between 267 bacterial isolates obtained from cucumber rhizosphere inoculated with *B. velezensis* SQR9. Leaf labels indicate representative sequence IDs. Labels marked red represent *Pseudomonas* isolates. Inner ring represents the medium on which isolates were originally obtained. Outer strips indicate phylum-level taxonomy of isolates. Annotation texts are the ASVs matching to the isolates with highest sequence similarity.

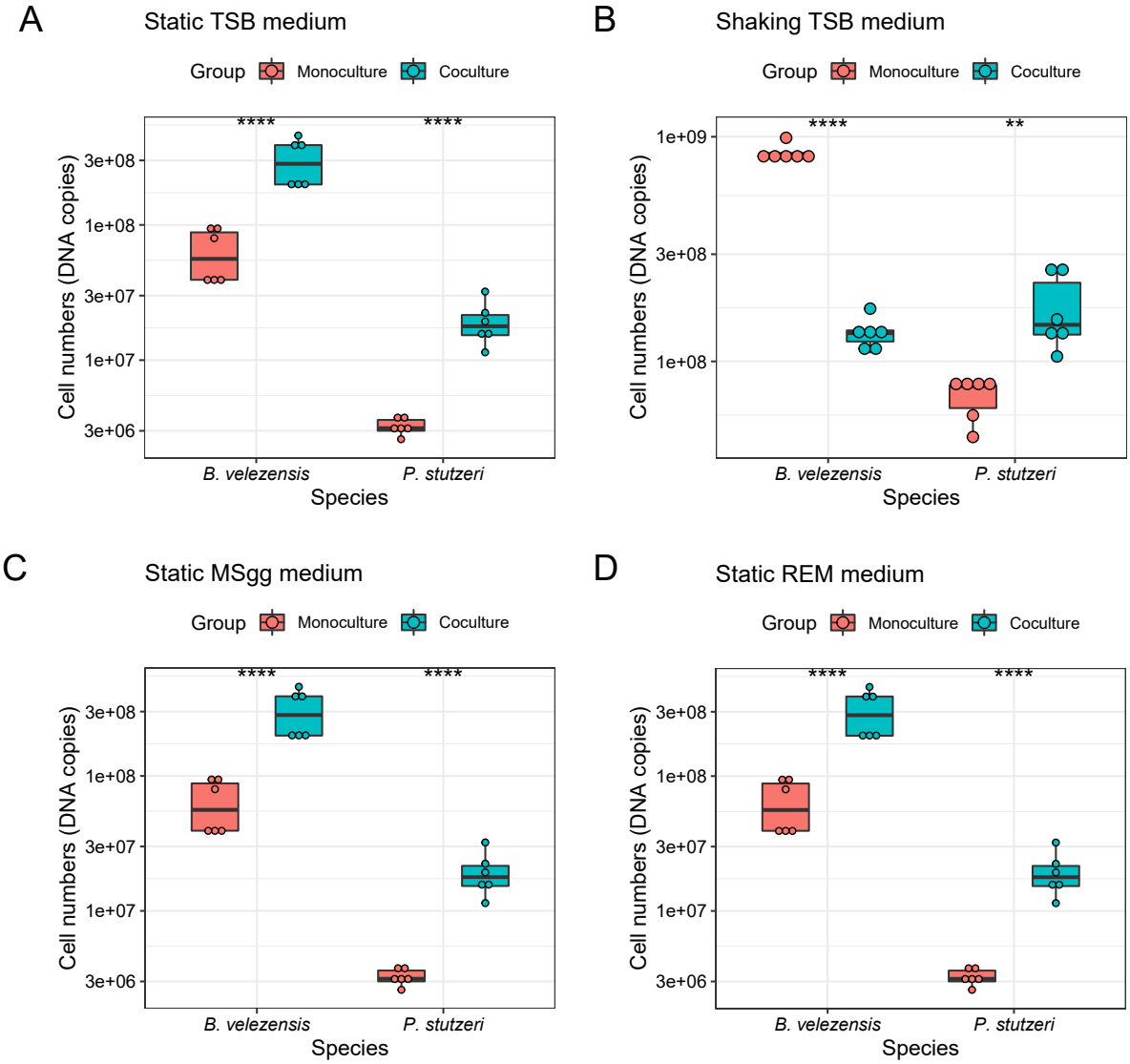

Fig. S2 Cell numbers of *B. velezensis* SQR9 and *P. stutzeri* XL272 in monoculture and coculture. They showed facilitation in static TSB and REM medium, while competition in shaking TSB medium and static MSgg medium. **(A)** Static TSB medium. **(B)** Shaking TSB medium. **(C)** Static MSgg medium. **(D)** Static REM medium. Asterisks indicate statistical significance according to unpaired student's *t* test via R: $^{**}p < 0.01$; $^{***}p < 0.001$.

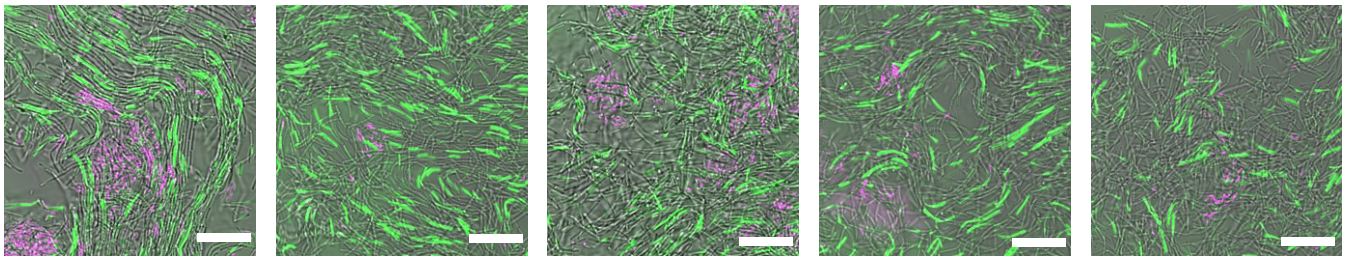

Fig. S3 Cocultured biofilm. Biofilm formed by *P. stutzeri* XL272 (magenta) and *B. velezensis* SQR9 (green) were viewed under the CLSM. These photos showed the heterogeneity of biofilm. Scale bar represents 20 μm.

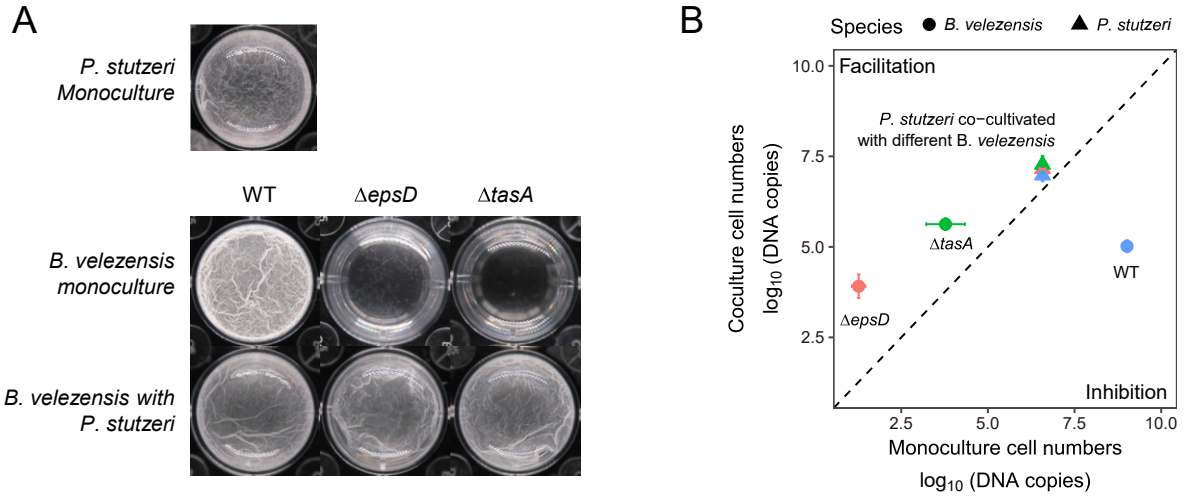

Fig. S4 EPS and TasA are essential for interaction in MSgg minimal medium. **(A)** Formation of pellicle biofilms by the mutants deficient in biosynthesis of exopolysaccharide EPS (Δ*epsD*) and TasA protein fibers (Δ*tasA*). Cells were incubated in MSgg at 30 °C for 24h before images were taken. Well diameter is 15.6 mm. **(B)** Cell numbers in dual species biofilm. Circle dots represent *B. velezensis*, triangles represent *P. stutzeri*. Colors indicate *B. velezensis* strain genotypes in coculture: WT (blue), Δ*epsD* (pink), Δ*tasA* (green). Data presented are the mean ± s.d. (n = 6).

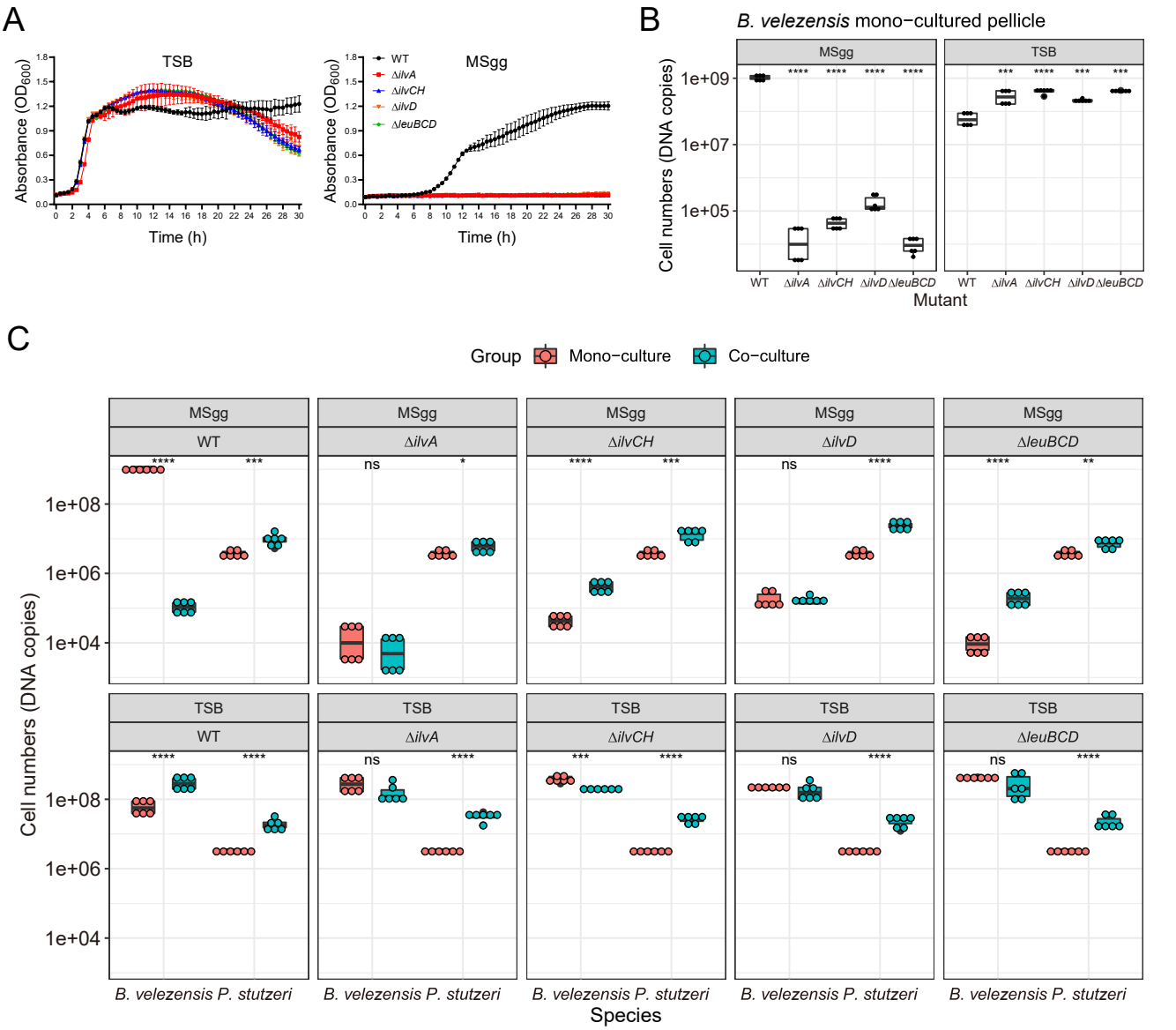

Fig. S5 Ability to synthesize BCAAs are essential for *B. velezensis* SQR9 to survive in minimal medium but dispensable in rich medium. (A) Growth curves of *B. velezensis* BCAAs biosynthetic mutants. **(B)** Cell numbers of *B. velezensis* mutants in monocultured pellicle. Asterisks indicate statistically significant ($p < 0.01$) compared with wildtype according to unpaired student's t test via R. Well diameter is 15.6 mm. **(C)** Cell numbers of dual species biofilm. Upper title indicates the medium type, lower title indicates *B. velezensis* mutants. Data presented are the values of six replicates. Asterisks indicate statistical significance according to unpaired student's t test via R : *$p < 0.05$; **$p < 0.01$.

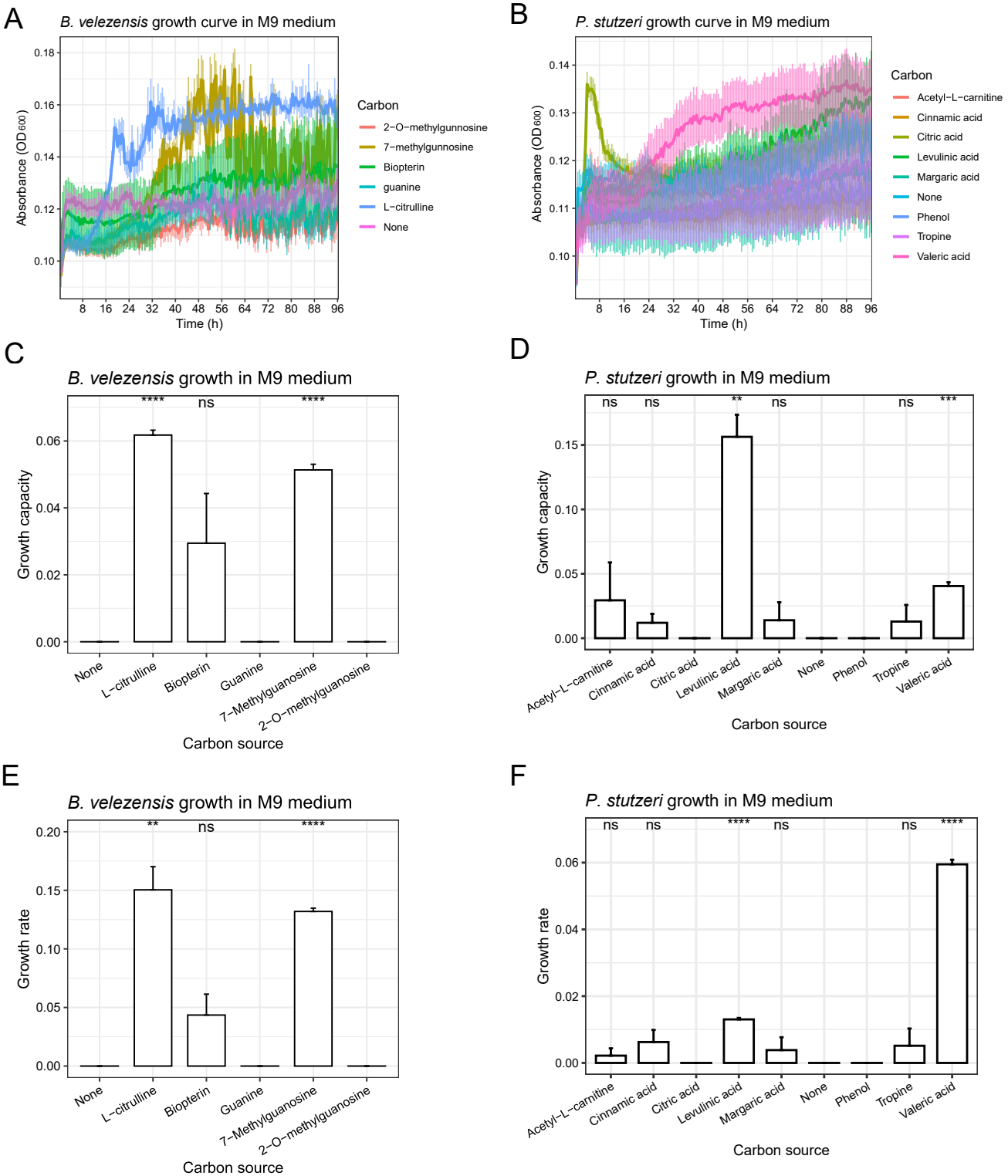

Fig. S6 Growth kinetics on individual compounds. (A&B) Growth curves of *B. velezensis* SQR9 and *P. stutzeri* XL272 grown in M9 medium with corresponding compounds as sole carbon sources. **(C&D)** Growth capacity is the maximum population size. The value is the maximum OD_{600} subtract the initial OD_{600}. **(E&F)** Growth rate is the slope of the growth curve at the exponential phase. Data presented are the mean ± s.d. (n = 5). Error bars represent standard deviations. Asterisks indicate statistical significance according to unpaired student's *t* test via R : $^{**}p < 0.01$, $^{***}p < 0.001$.

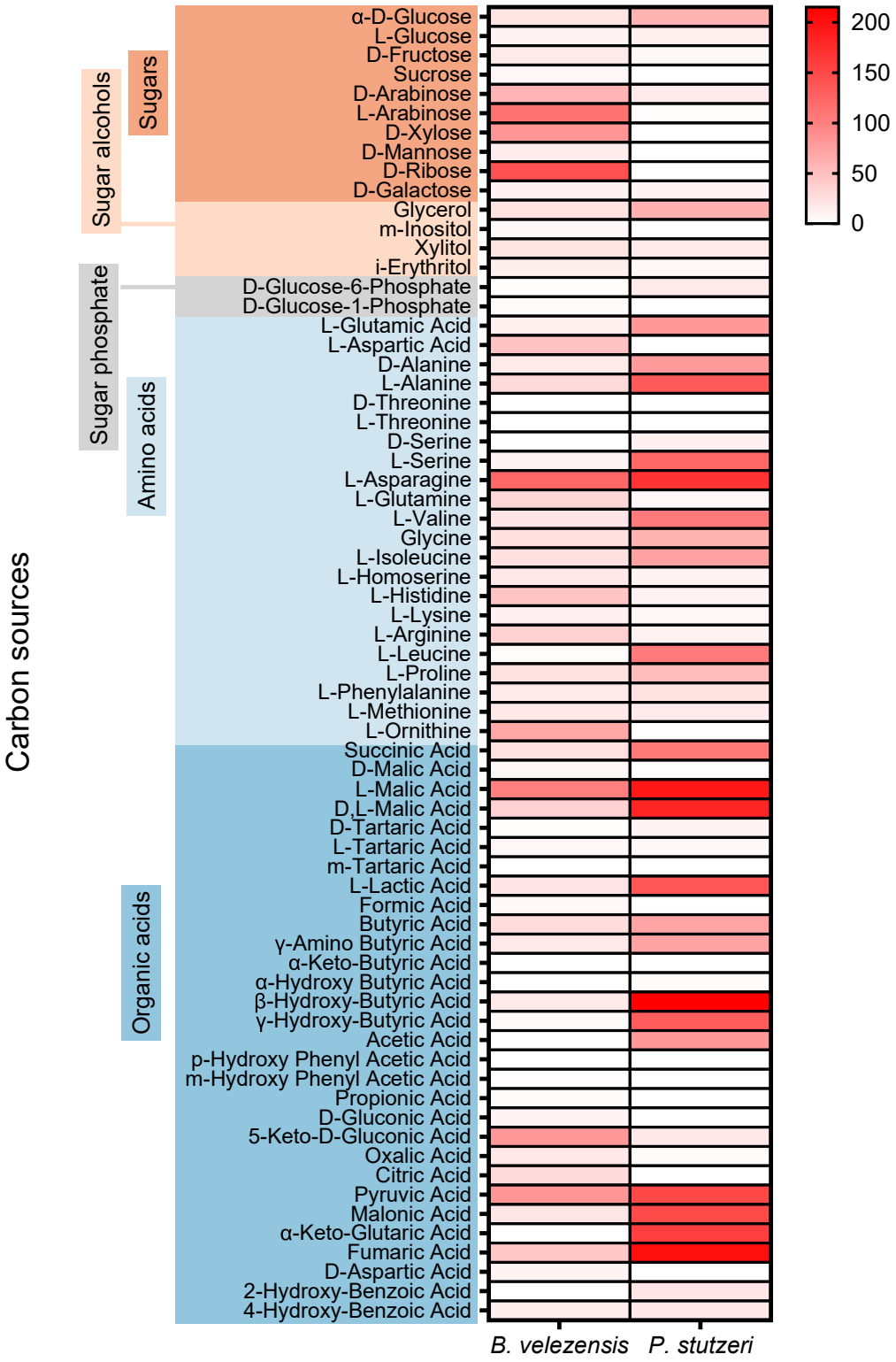

Fig. S7 Differential utilization of root secreted carbon sources. Heatmap colour indicates utilizing ability determined by Phenotype Microarray(PM) technology. Root secreted carbon sources come from the [1].

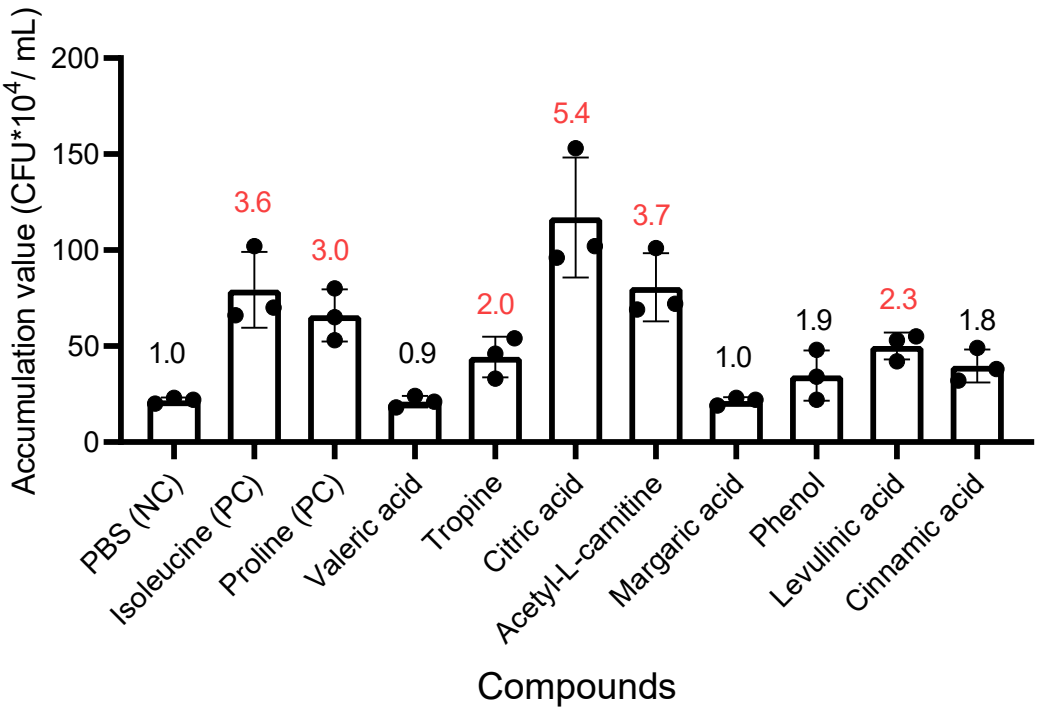

Fig. S8 Chemotactic response of *P. stutzeri* XL272 towards several compounds secreted by *B. velezensis* SQR9. Accumulation of bacteria in the capillaries was calculated as the average from the CFUs obtained in triplicate plates, and the results were expressed as the mean of at least three separate capillary assays for each determination. Error bars indicate the standard deviations based on three different replicates. Negative control is solvent PBS. Positive controls are isoleucine and proline. Numbers on top of each bar indicate the relative chemotactic indexes (RCI), RCI >= 2 indicates strong chemotactic response.

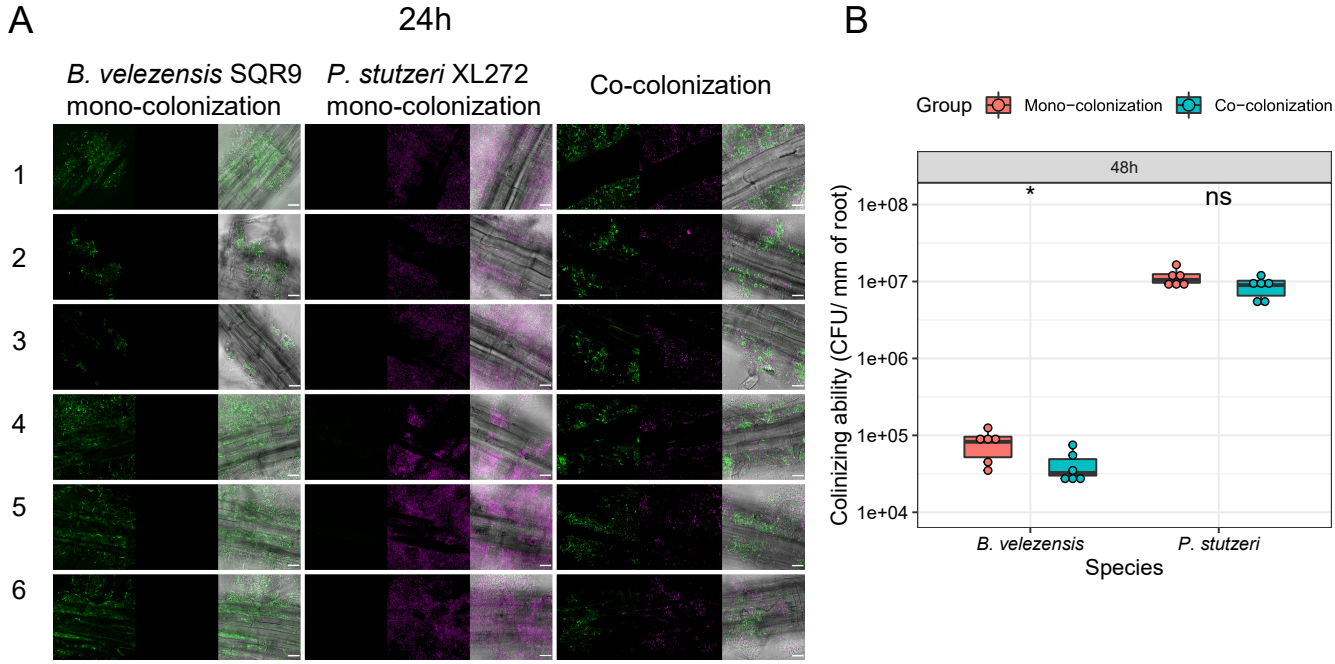

Fig. S9 Colonization on *A. thaliana* roots. (A) Colonization at 24h was visualized using CLSM. *B. velezensis* SQR9 (GFP, colored green), *P. stutzeri* XL272 (DsRed, false colored magenta). Scale bar represents 20 µm. **(B)** Colonization at 48 h. Colonizing ability of the bacteria were measured as CFUs per millimeter of root (n = 6) (see Methods). The samples were collected at 48 h. The colonizing ability of mono-colonization is compared with co-colonization. Asterisks indicate statistically significant ($p < 0.01$) according to unpaired student's t test via R.

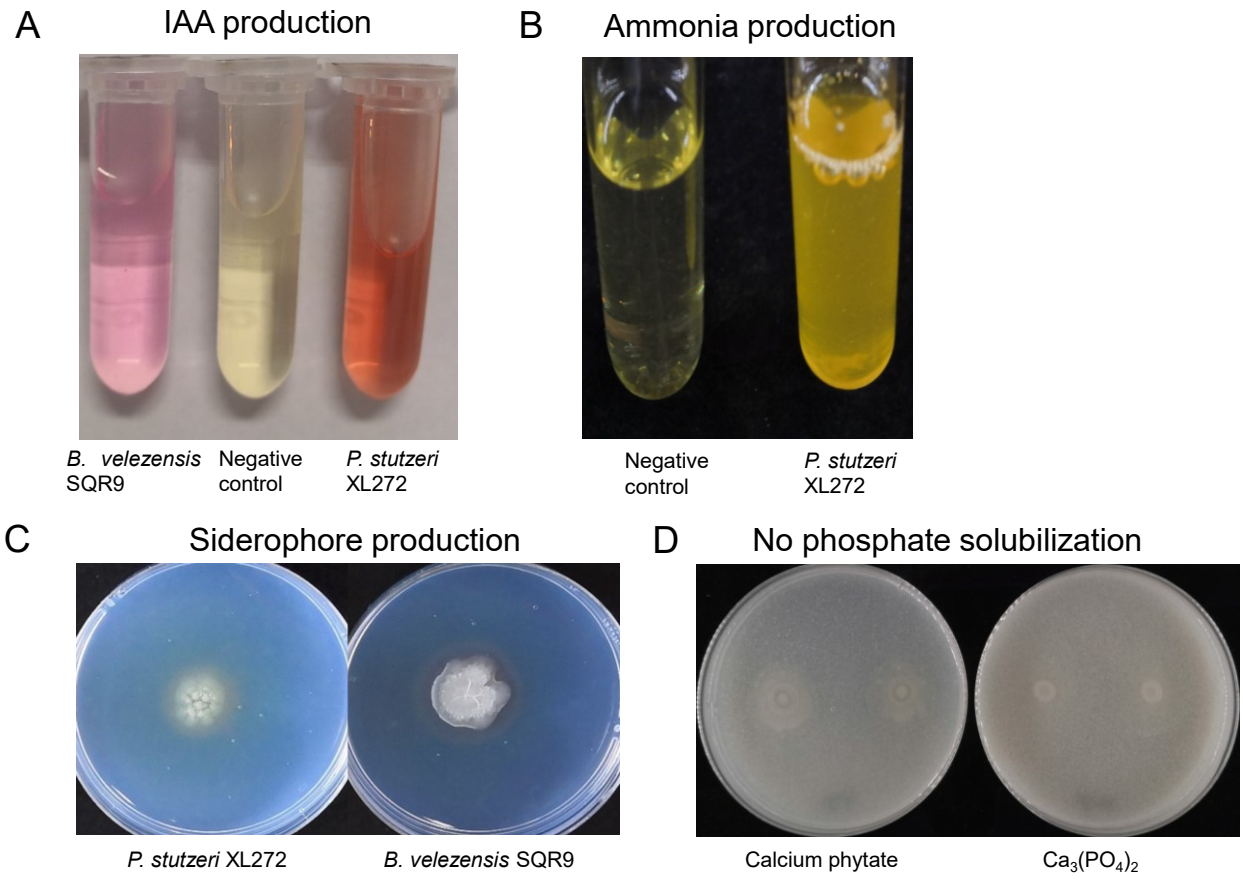

Fig. S10 Plant-growth promoting traits of *P. stutzeri* XL272. (A) *P. stutzeri* XL272 is capable of IAA production at a concentration of 12.23 μg/mL. **(B)** *P. stutzeri* XL272 is tested positive for production of ammonia. **(C)** *P. stutzeri* XL272 is capable of siderophore production. (**D)** *P. stutzeri* XL272 can not solubilize phosphate. Plate diameter is 9 cm.

Supplementary tables

Table S1: Strains and plasmids used in this study:

Strains	Description	Source or reference
***Bacillus velezensis* SQR9**		
SQR9	Wild type	Lab sotck
SQR9GFP	Wild typeSQR9::gfp; cm^R	Lab sotck
Δ*eps*	*eps*::cm^R	Lab sotck
Δ*tasA*	*tasA*::cm^R	Lab stock [2]
Δ*ilvA*	*ilvA*::cm^R	This study
Δ*ilvCH*	*ilvCH*::cm^R	This study
Δ*ilvD*	*ilvD*::cm^R	This study
Δ*leuBCD*	*leuBCD*::cm^R	This study
***Pseudomonas stutzeri* XL272**		
XL272	Natural isolate, wild type	This study
XL272-DsRed	*attTn7*::$P_{A1/04/03}$-*dsRed*	This study
gm^R: gentamicin resistance, cm^R: chloramphenicol resistance		
Other *Pseudomonas* spp.		
Pseudomonas nitroreducens XL271	Natural isolate, wild type	This study
Pseudomonas stutzeri XL273	Natural isolate, wild type	This study
Pseudomonas monteilii XL274	Natural isolate, wild type	This study
Pseudomonas pseudoalcaligenes XL275	Natural isolate, wild type	This study
Pseudomonas resinovorans XL278	Natural isolate, wild type	This study
Plasmids		
pminiTn7(Gm)PA1/04/03-DsRedExpress-a	Mini-transposon delivery plasmid	[3]
pUX-BF13	Mini-transposon helper plasmid	[3]

Table S2: Primers used in this study:

Primers	Sequence (from 5' to 3')	Experimental purpose
S_1F	CAAATTGAGAAAAAGCTAAGCAAACCGA	To quantifiy the cell numbers of *B. velezensis* SQR9
S_1R	TCTTCCCCGGTTAATTCGTATATGGTA	To quantifiy the cell numbers of *B. velezensis* SQR9
P_54F	CGCGCCCAAACTAGCAACTTAGACA	To quantifiy the cell numbers of *P. stutzeri* XL272
P_54R	CAATACGGGGAATATTTCAGAACTCGG	To quantifiy the cell numbers of *P. stutzeri* XL272
IlvA_UF	CGTCCAGGTTCTCAGCCTT	To amplify the upstream region of *ilvA* gene of *B. velezensis* SQR9

IlvA_UR	CGTTACGTTATTAGTTATCCGCATTGGTCGGCATTG	To amplify the upstream region of *ilvA* gene of *B. velezensis* SQR9
A_Spc-UF	CAATGCCGACCAATGCGGATAACTAATAACGTAACG	Cloning
A_Spc-UR	CGGGAAACCATGCGCAGCGTATAATGTATGCTATA	Cloning
IlvA_DF	TATAGCATACATTATACGCTGCGCATGGTTTCCCG	To amplify the downstream region of *ilvA* gene of *B. velezensis* SQR9
IlvA_DR	TAGCACGCCGCATTTTGAAG	To amplify the downstream region of *ilvA* gene of *B. velezensis* SQR9
IlvA_F	ACCGGTCAGAAAATCCCAGTT	To confirm deletion of *ilvA* gene in *B. velezensis* SQR9
IlvA_R	GGGATAAATCGTGCTGCCGG	To confirm deletion of *ilvA* gene in *B. velezensis* SQR9
IlvCH_UF	CTGACATGAAGCGCAACGGC	To amplify the upstream region of *ilvCH* gene of *B. velezensis* SQR9
IlvCH_UR	CGTTACGTTATTAGTTATCAATGCGATCAATGCAAGCG	To amplify the upstream region of *ilvCH* gene of *B. velezensis* SQR9
CH_Spc-UF	CGCTTGCATTGATCGCATTGATAACTAATAACGTAACG	Cloning
CH_Spc-UR	AGCATCACTGTAGGCCACACCGTATAATGTATGCTATA	Cloning
IlvCH_DF	TATAGCATACATTATACGGTGTGGCCTACAGTGATGCT	To amplify the downstream region of *ilvCH* gene of *B. velezensis* SQR9
IlvCH_DR	GCAAATACCGGTAGCGCACA	To amplify the downstream region of *ilvCH* gene of *B. velezensis* SQR9
IlvCH_F	CTGACAAGATCACTCGTCCGC	To confirm deletion of *ilvCH* gene in *B. velezensis* SQR9
IlvCH_R	CATGAGATCAGCCTTCCGGG	To confirm deletion of *ilvCH* gene in *B. velezensis* SQR9
IlvD_UF	CTTTGCGCGGAACATCGC	To amplify the upstream region of *ilvD* gene of *B. velezensis* SQR9
IlvD_UR	CGTTACGTTATTAGTTATGGACGTACAAGTGCCTGAAGAA	To amplify the upstream region of *ilvD* gene of *B. velezensis* SQR9
D_Spc-UF	TTCTTCAGGCACTTGTACGTCCATAACTAATAACGTAACG	Cloning
D_Spc-UR	GGCGGTGTGCCTTTTGAATCGTATAATGTATGCTATA	Cloning
IlvD_DF	TATAGCATACATTATACGATTCAAAAGGCACACCGCC	To amplify the downstream region of *ilvD* gene of *B. velezensis* SQR9
IlvD_DR	CTGACAGAAGCAGCCGCACA	To amplify the downstream region of *ilvD* gene of *B. velezensis* SQR9
IlvD_F	CGGTTTGGAGCTGCTCCATT	To confirm deletion of *ilvD* gene in *B. velezensis* SQR9
IlvD_R	CTTCAGGCCGCCTACAGTGA	To confirm deletion of *ilvD* gene in *B. velezensis* SQR9
LeuBCD_UF	GATGTGCGCCCGGATCTTGA	To amplify the upstream region of *leuBCD* gene of *B. velezensis* SQR9
LeuBCD_UR	CGTTACGTTATTAGTTATAGTGGAAAAAACTCGCCGGT	To amplify the upstream region of *leuBCD* gene of *B. velezensis* SQR9
Spc-UF	ACCGGCGAGTTTTTTCCACTATAACTAATAACGTAACG	Cloning
Spc-UR	AAGCGTTGCCGAACAATTCGCGTATAATGTATGCTATA	Cloning

LeuBCD_DF	TATAGCATACATTATACG CGAATTGTTCGGCAACGCTT	To amplify the downstream region of *leuBCD* gene of *B. velezensis* SQR9
LeuBCD_DR	AGCCGGGTAAAGCTCTCAGC	To amplify the downstream region of *leuBCD* gene of *B. velezensis* SQR9
LeuBCD_F	TCGGAACCGCGGTCATTGAA	To confirm deletion of *leuBCD* gene in *B. velezensis* SQR9
LeuBCD_R	AACCTGCCGGACACTGTAGG	To confirm deletion of *leuBCD* gene in *B. velezensis* SQR9
IlvA_SF	CCCAAGTGGAACTGTTCGG	To quantify the relative expression of *ilvA* gene in *B. velezensis* SQR9
IlvA_SR	AAATTTCGATGGCAGCCGT	To quantify the relative expression of *ilvA* gene in *B. velezensis* SQR9
IlvC_SF	GTGCCTCCTGCTGATGTG	To quantify the relative expression of *ilvC* gene in *B. velezensis* SQR9
IlvC_SR	GTTTCAAGAACGCCCGCA	To quantify the relative expression of *ilvC* gene in *B. velezensis* SQR9
IlvD_SF	GGCTCACTGGTTTGACGG	To quantify the relative expression of *ilvD* gene in *B. velezensis* SQR9
IlvD_SR	TTTCCCGGCTTGATATGCG	To quantify the relative expression of *ilvD* gene in *B. velezensis* SQR9
IlvE_SF	GGGTGTTGATGTTCCGTCC	To quantify the relative expression of *ilvE* gene in *B. velezensis* SQR9
IlvE_SR	GTCGCAATTACAAACGGACG	To quantify the relative expression of *ilvE* gene in *B. velezensis* SQR9
IlvH_SF	GGATCACCGGCCTGTTTACA	To quantify the relative expression of *ilvH* gene in *B. velezensis* SQR9
IlvH_SR	TGCCAGCTCACGCTGTAC	To quantify the relative expression of *ilvH* gene in *B. velezensis* SQR9
leuA_SF	GCCCGATCTGTCAAAGGTG	To quantify the relative expression of *leuA* gene in *B. velezensis* SQR9
leuA_SR	GCTGACCATTGAACGATCGG	To quantify the relative expression of *leuA* gene in *B. velezensis* SQR9
leuB_SF	AAGATCCGACGCCATCCT	To quantify the relative expression of *leuB* gene in *B. velezensis* SQR9
leuB_SR	TTACAAAGTCGACGCCGCT	To quantify the relative expression of *leuB* gene in *B. velezensis* SQR9
leuC_SF	GGTCTGACACTTCCGGGGA	To quantify the relative expression of *leuC* gene in *B. velezensis* SQR9
leuC_SR	TGACATCCTTCGCGGTCAC	To quantify the relative expression of *leuC* gene in *B. velezensis* SQR9
leuD_SF	CGGTGAGCCTGATCCTGAA	To quantify the relative expression of *leuD* gene in *B. velezensis* SQR9
leuD_SR	CGGATGGGAAGCATACCGTT	To quantify the relative expression of *leuD* gene in *B. velezensis* SQR9
RecA_SF	AAAAAACAAAGTCGCTCCTCCG	To amplify the *recA* reference gene in *B. velezensis* SQR9
ReckA_SR	CGATATCCAGTTCAGTTCCAAG	To amplify the *recA* reference gene in *B. velezensis* SQR9
IlvA_PF	GCTGAAGCTGGTCGAGGAGAA	To quantify the relative expression of *ilvA* gene in *P. stutzeri* XL272
IlvA_PR	GCTGACGCAGGATTTCCATCG	To quantify the relative expression of *ilvA* gene in *P. stutzeri* XL272
IlvC_PF	CGCCAAGATGTTCATCACCGAA	To quantify the relative expression of *ilvC* gene in *P. stutzeri* XL272

IlvC_PR	AGCTTCTCGCCGACCACTTC	To quantify the relative expression of *ilvC* gene in *P. stutzeri* XL272
IlvD_PF	CCACATGGAAGACGTGCAC	To quantify the relative expression of *ilvD* gene in *P. stutzeri* XL272
IlvD_PR	TCCCATTCGGCGATGGCA	To quantify the relative expression of *ilvD* gene in *P. stutzeri* XL272
IlvE_PF	CAACCTGAAGGTGCACGTG	To quantify the relative expression of *ilvE* gene in *P. stutzeri* XL272
IlvE_PR	GCGGGTCATGGTGATGTTG	To quantify the relative expression of *ilvE* gene in *P. stutzeri* XL272
IlvH_PF	GGCCACGAGGAGGTGATC	To quantify the relative expression of *ilvH* gene in *P. stutzeri* XL272
IlvH_PR	GCCGGTAGCCTTGACCTT	To quantify the relative expression of *ilvH* gene in *P. stutzeri* XL272
leuA_PF	GCCTACCAGCCGCAGATCAT	To quantify the relative expression of *leuA* gene in *P. stutzeri* XL272
leuA_PR	CTCCAGCAGCGGAATGAACAC	To quantify the relative expression of *leuA* gene in *P. stutzeri* XL272
leuB_PF	ATATCCGCAACTGGCCGA	To quantify the relative expression of *leuB* gene in *P. stutzeri* XL272
leuB_PR	CATTCTCGAGCACCTTGC	To quantify the relative expression of *leuB* gene in *P. stutzeri* XL272
leuC_PF	GCTTGCCGATGTAGTCCGAAAC	To quantify the relative expression of *leuC* gene in *P. stutzeri* XL272
leuC_PR	GCACTGAAGACCACTCCGTTC	To quantify the relative expression of *leuC* gene in *P. stutzeri* XL272
leuD_PF	AGATTCACGGTGCGGATGC	To quantify the relative expression of *leuD* gene in *P. stutzeri* XL272
leuD_PR	TTGTTGCGCTCGCCCTGA	To quantify the relative expression of *leuD* gene in *P. stutzeri* XL272
RpoD_PF	TCCTCAGCGGCTACATCG	To amplify the *rpoD* reference gene in *P. stutzeri* XL272
RpoD_PR	TTATCGTCGGCAACCGGC	To amplify the *rpoD* reference gene in *P. stutzeri* XL272

Table S3: Water-solubility of the compounds and concentration provided as sole carbon source in M9 medium:

Compound	**Water Solubility**	**Concentration**
Glucose	Soluble	10 g/L
L-citrulline	Soluble	10 g/L
Biopterin	0.1 mg/L	0.1 mg/L
Guanine	Insoluble	< 0.1 mg/L
7-methylguanosine	5.61 mg/mL	5.61 mg/mL
2'-O-methylgusnosine	Insoluble	< 0.1 mg/L
Acetyl-L-carnitine	0.355 mg/mL	0.355 mg/mL
Valeric acid	49.7 mg/L	49.7 mg/L
Tropine	Soluble	10 g/L
Cinnamic acid	0.62 g/L	0.62 g/L
Phenol	Soluble	10 g/L
Margaric acid	Insoluble	< 0.1 mg/L
Citric acid	Soluble	10 g/L
Levulinic acid	Soluble	10 g/L

References

1. Sasse J, Martinoia E, Northen T. Feed your friends: do plant exudates shape the root microbiome? *Trends Plant Sci* 2018. **23**: 25–41

2. Xu Z, Mandic-Mulec I, Zhang H, Liu Y, Sun X, Feng H, et al. Antibiotic bacillomycin D affects iron acquisition and biofilm formation in *Bacillus velezensis* through a Btr-mediated FeuABC-dependent pathway. *Cel Rep* 2019; **29**: 1192-1202.

3. Lambertsen L, Sternberg C, Molin S. Mini-Tn7 transposons for site-specific tagging of bacteria with fluorescent proteins. *Environ Microbiol* 2004; **6**: 726–732.