

Supplementary Table S1: Bacterial strains and plasmids used in this study

Bacteria and Plasmids	Characteristics†	Source/Reference
Bacteria		
<i>E. coli</i> EC1000	Derivative of <i>E. coli</i> MC1000 in which <i>repA</i> is integrated in chromosome	(1)
<i>L. reuteri</i> ATCC PTA 6475	Human breast milk isolate	BioGaia AB
VPL4073	In-frame deletion of 2 kb <i>pduCDE</i> in <i>L. reuteri</i> 6475	This work
<i>L. reuteri</i> R2lc	Rat gastrointestinal isolate	(2)
VPL4192	In-frame deletion of 870 bp <i>araT</i> in <i>L. reuteri</i> R2lc	This work
<i>L. reuteri</i> DSM 17938	Plasmid-cured derivative of <i>L. reuteri</i> ATCC 55730	BioGaia AB, (3)
VPL3565	<i>L. reuteri</i> DSM 17938 harboring pVE6007	This work
VPL4171	In-frame deletion of 2.2 kb <i>sdpA</i> in <i>L. reuteri</i> DSM 17938	This work
VPL4176	In-frame deletion of 5.3 kb <i>sdpB</i> in <i>L. reuteri</i> DSM 17938	This work
<i>L. rhamnosus</i> GG	Human fecal isolate	ATCC
VPL4185	In-frame deletion of 1.5 kb <i>acs1</i> in <i>L. rhamnosus</i> GG	This work
<i>L. casei</i> BFLM218	Human fecal isolate	(4)
VPL4013	Insertion of 66 bp 3x-FLAG tag <i>prtP</i> gene in <i>L. casei</i> BFLM218	This work
<i>L. brevis</i> ATCC 8287	Fermented olives isolate	ATCC
VPL3978	<i>L. brevis</i> harboring pVPL3933	This work
<i>L. fermentum</i> ATCC 14931	Fermented beets isolate	ATCC
VPL3950	<i>L. fermentum</i> harboring pVPL3933	This work
<i>L. plantarum</i> ATCC BAA-793	Human saliva isolate	ATCC
VPL3938	<i>L. plantarum</i> harboring pVPL3933	This work
<i>L. salivarius</i> CCUG 47825	Human blood isolate	CCUG
VPL3941	<i>L. salivarius</i> harboring pVPL3933	This work
<i>L. sakei</i> ATCC 15521	Starter of sake	ATCC
VPL3952	<i>L. sakei</i> harboring pVPL3933	This work
<i>L. gasseri</i> ATCC 33323	Human intestinal isolate	ATCC
<i>Fructobacillus fructosus</i> DSM 20349	Flowers isolate	DSMZ
<i>Leuconostoc mesenteroides</i> DSM 20346	Hansen's dried starter powder	DSMZ
<i>Pediococcus acidilactici</i> DSM 20284	Barley isolate	DSMZ
<i>Weissella paramesenteroides</i> DSM 20288	Meat isolate	DSMZ
<i>Lactococcus lactis</i> subsp. <i>cremoris</i> NZ9000	Dairy starter, derivative of MG1363, <i>pepN::nisRK</i>	(5)
Plasmids		
pOR119	Em ^R , <i>repA</i> -	(6)
pSIP411	Em ^R , Sakacin-P based expression vector	(7)
pVPL2042	Em ^R , pNZ8048 derivative. Cm marker was replaced by Em marker	Van Pijkeren Lab stock
pVPL3002	pOR119 harboring <i>L. reuteri</i> derived <i>ddlF258Y</i>	This work
pVPL3010	Em ^R , derivative of vector pVPL3002 in which the <i>L. rhamnosus</i> GG Δ <i>acs1</i> deletion cassette was cloned in the MCS site	This work
pVPL3125	Em ^R , derivative of vector pVPL3002 in which 2kb 5'- and 3' flanking sequence required for 3x-FLAG tag insertion in <i>prtP</i> of <i>L. casei</i> BFLM218 was cloned in the MCS site	This work
pVPL3137	Em ^R , derivative of pVPL3125 in which protein encodes 3x-FLAG tag was cloned in between the integration cassette of pVPL3125	This work
pVPL3478	Em ^R , derivative of vector pVPL3002 in which the <i>L. reuteri</i> 6475 <i>pduCDE</i> deletion cassette was cloned in the MCS site	This work
pVPL3612	Em ^R , derivative of vector pVPL3002 in which the <i>L. reuteri</i> 17938 <i>sdpA</i> deletion cassette was cloned in the MCS site	This work
pVPL3694	Em ^R , derivative of vector pVPL3002 in which the <i>L. reuteri</i> R2lc <i>araT</i> deletion cassette was cloned in the MCS site	This work
pVPL3762	Em ^R , derivative of vector pVPL3002 in which the <i>L. reuteri</i> 17938 <i>sdpB</i> deletion cassette was cloned in the MCS site	This work
pVPL3859	Em ^R , derivative of vector 2042 in which the <i>L. plantarum</i> BAA-793 <i>ddl</i> was inserted in the MSC site	This work
pVPL3862	Em ^R , derivative of pVPL2042 in which <i>ddlF258Y</i> was cloned in the backbone of pVPL2042	This work

Plasmid	Characteristics†	Source/Reference
pVPL3925	Em ^R , derivative of vector 2042 in which the <i>L. plantarum</i> BAA-793 <i>ddlF260Y</i> was inserted in the MSC site	This work
pVPL3933	Em ^R , derivative of pSIP411 in which <i>ddlF260Y_{plantarum}</i> was cloned under the control of sakacin-based inducible promoter	This work

VPL: Van Pijkeren Lab strain identification number. pVPL: Van Pijkeren Lab plasmid identification number.

†: *pduCDE*: glycerol dehydratase large subunit PduC, glycerol dehydratase medium subunit PduD, glycerol dehydratase small subunit PduE (BAG26151, BAG26150.1, BAG26149.1); *araT*: aromatic amino acid aminotransferase (MG822655); *sdpA*: bifunctional phosphodiesterase (WP_003672341.1); *sdpB*: hypothetical protein and LPXTG-motif cell wall anchor domain protein (WP_013923860.1 , AEI56985.1); *acs1*: acyl-CoA synthetase (WP_014570113.1); Em^R: erythromycin-resistant; Cm^R: chloramphenicol resistant. The accession number listed can be found on <https://www.ncbi.nlm.nih.gov>. *: ATCC: American Type Culture Collection; DSMZ: German Collection of Microorganisms and Cell Cultures; CCUG: Culture Collection University of Gothenburg.

Table S2. Oligonucleotides used in this study

oligo name	sequence (5'-3')†	Target/comment#
oVPL46	/5phos/aggaaatgcatattcgaactggag	Fwd, amplifies <i>ddlF258Y</i> of <i>L. reuteri</i> 6475 including native promoter region
oVPL47	/5phos/acattgtactcacaacatcctaacc	Rev, amplifies <i>ddlF258Y</i> of <i>L. reuteri</i> 6475
oVPL48	tatccgctcacaattccacac	Fwd, amplified the backbone of pORI19
oVPL49	acaattcacacaggaacacgc	Rev, amplified the backbone of pORI19
oVPL52	tcggaattctgattcactcacacgcatgg	Fwd, 3' u/s flanking region of <i>L. rhamnosus</i> GG
oVPL53	cagtcgtaactacgctcttctgctcattgattcaggactc	Rev, 3' u/s flanking region of <i>L. rhamnosus</i> GG
oVPL54	agtcctgaatcatgagcaagaagcgaagttacgactg	Fwd, 5' u/s flanking region of <i>L. rhamnosus</i> GG
oVPL55	agctctagaaagcgtccttacacgcagatc	Rev, 5' u/s flanking region of <i>L. rhamnosus</i> GG
oVPL88	catggtagtgccctctataattta	Rev, starting at ATG of NcoI site of pNZ8048
oVPL89	tagttctagagagctcaagcttcc	Fwd, starting at 4bp upstream of XbaI site of pNZ8048
oVPL94	agatctagcttataactatactgacaatag	Fwd, for pVPL3862 screening
oVPL146	tctatccaactggctcaagg	Internal oligo for d/s SCO screening of pVPL3010 chromosomal integration
oVPL147	actggaagatccgccaatac	Internal oligo for u/s SCO screening of pVPL3010 chromosomal integration
oVPL169	ggtttcccagtcacgagctgtaaaacgacggccagtgaaattcgagctcagcgtccttacacgcagatccctg	Gibson oligo, combined with oVPL170 for overlap extension PCR to construct <i>acs1</i> deletion cassette
oVPL170	tcgaaatgcatcttctcacaattcacacaggaacagctatgacctgagattcacttcacacgcatgggc	Gibson oligo, combined with oVPL169 for overlap extension PCR to construct <i>acs1</i> deletion cassette
oVPL171	caacaaccataaacgcttcaccagctcagggatctgctgtaaggacgctgagctcgaattcactggccgtcg	Amplifies the backbone of pVPL3002 for Gibson assembly
oVPL172	tcagcaccagccctgtcatgaacatgaagcccatgctgtaagtgaaatctcatggctatagctgttctctg	Amplifies the backbone of pVPL3002 for Gibson assembly
oVPL187	taccgagctcgaattcactgg	Rev, amplifies pVPL3002 backbone
oVPL188	atcctctagagtcgacctgc	Fwd, amplifies pVPL3002 backbone
oVPL192	ttatcgtcacgagccaacac	Fwd, combined with oVPL193 for screening pVPL3010 chromosomal integration and screening <i>acs1</i> deletion
oVPL193	tcagttggtcaccgaaacag	Rev, combined with oVPL192 for screening pVPL3010 chromosomal integration and screening <i>acs1</i> deletion
oVPL194	tgtgctgaaggcgattaag	Fwd, for pVPL3125 screening
oVPL380	/5phos/ttcttgtaaaagctacttctcagtcac	Fwd, amplifies the integration cassette of pVPL3137 from <i>L. casei</i> BFLM218
oVPL381	/5phos/atcgtgttgacaatgatcaagcc	Rev, amplifies the integration cassette of pVPL3137 from <i>L. casei</i> BFLM218
oVPL382	agtcggataataaaacttccgcaatg	Fwd, combined with oVPL383 for amplifying the backbone of pVPL3125, which can be used for Gibson assembly with insertion cassette (oVPL384-oVPL385)
oVPL383	gatgctaaggccaactcgatg	Rev, combined with oVPL193 for screening pVPL3010 chromosomal integration and screening <i>acs1</i> deletion
oVPL384	agttaaacagtcacattggcgaaagttattatccgactgattataaggatcatgatggcgattataaggatcatgatcattataaggatgatgatgataag gatgctaaggccaactcgatggcgaatgcaagccgat	Combined with oVPL385 for self-ligation to form double strand fragment (3x-FLAG tag) which can be cloned in the backbone of pVPL3125

oligo name	Sequence (5'-3') [†]	Target/comment [#]
oVPL385	atacggcttgacacatcgccatcgagttggccttagcatccttatcatcatccttataatcgatcatcatgatcctataatcgccatcatgatcctataatc agtcggataataaaccttcccaatggactgttttaact	Combined with oVPL384 for self-ligation to form double strand fragment (3x-FLAG tag) which can be cloned in the backbone of pVPL3125
oVPL386	ttggcgaaagtttattatccgactgattata	Combined with oVPL387-oVPL388 to screen pVPL3137
oVPL387	aaactaaaggatcatcactcgcaaatcag	Combined with oVPL386-oVPL388 to screen pVPL3137
oVPL388	atcctaaggacatgttgaggacatcg	Combined with oVPL386-oVPL387 to screen pVPL3137
oVPL399	ggctaaaatctccttgaatagtatattatag	Fwd, amplifies the backbone of pSIP411
oVPL400	taactagactcgaggaattcggtac	Rev, amplifies the backbone of pSIP411
oVPL504	agtttattatccgactgattataaggatc	Fwd, combined with oVPL506-oVPL507 for <i>L. casei</i> BFLM218 3x-FLAG tag insertion SCO screening
oVPL505	ttagcatccttatcatcatc	Rev, combine with oVPL506-oVPL507 for <i>L. casei</i> BFLM218 3x-FLAG tag insertion SCO screening
oVPL506	tagtgtaaccgatttccatagcc	Fwd, combined with oVPL507 and oVPL504 to screen <i>L. casei</i> BFLM218 3x-FLAG tag insertion screening
oVPL507	ttgaccggtttactggagagcc	Rev, <i>L. casei</i> BFLM218 3x-FLAG tag insertion screening
oVPL659	tgccccgttagtgaaagaag	Fwd, amplifies the pSIP411
oVPL660	attctgctcccccttatg	Rev, amplifies the pSIP411
oVPL703	tgctgcttttggctatcaa	Rev, for pVPL3862 screening
oVPL1335	ggttcttgaatgatgatgca	Fwd, 5' u/s flanking region of <i>L. reuteri</i> pduCDE
oVPL1336	acaacagccgaacgatttcc	Rev, 5' u/s flanking region of <i>L. reuteri</i> pduCDE
oVPL1337	tggtgctagcgaatggag	Fwd, 3' d/s flanking region of <i>L. reuteri</i> pduCDE
oVPL1338	tacgatctgccatttcaac	Rev, 3' d/s flanking region of <i>L. reuteri</i> pduCDE
oVPL1339	agtgtaaagttgaaaatggcaagatcgttaggtcttgaatgatgatgcattgcgtcc	LCR bridging oligo for pORI- <i>ddIF258Y::pduCDE</i> deletion cassette
oVPL1340	aaacgacggccagtgaaatcgagctggatgggtgtagcgaatggagataaccattgg	LCR bridging oligo for pORI- <i>ddIF258Y::pduCDE</i> deletion cassette
oVPL1341	agcgcctattggaaatcgttggctgtgtatcctctagagtcgacctgagcatgcaa	LCR bridging oligo for pORI- <i>ddIF258Y::pduCDE</i> deletion cassette
oVPL1342	agttgatgccggagtacaag	Fwd, u/s SCO screening of pVPL3478 chromosomal integration
oVPL1343	tgccgtggctcattgattc	Also for DCO screening of Δ <i>pduCDE</i> in <i>L. reuteri</i> 6475
oVPL1344	acattggtccagactcaccag	Rev, u/s SCO screening of pVPL3478 chromosomal integration
oVPL1345	atggctggagctgaagtagg	Fwd, d/s SCO screening of pVPL3478 chromosomal integration
oVPL1562	atatagtagttacgtaactcaccg	Rev, d/s SCO screening of pVPL3478 chromosomal integration
oVPL1563	tggtagattttgaacgagcattacc	Also for DCO screening of Δ <i>sdpA</i> in <i>L. reuteri</i> DSM 17938
oVPL1754	atccttgccgatgaatttcgattg	Fwd, for screening of Δ <i>sdpA</i> in <i>L. reuteri</i> DSM 17938
oVPL1755	caagaaatgaaagatgcttatgagc	Fwd, 5' u/s flanking region of <i>L. reuteri</i> R2lc <i>araT</i>
oVPL1756	agtagctggcatgataaattcctc	Rev, 5' u/s flanking region of <i>L. reuteri</i> R2lc <i>araT</i>
oVPL1757	tgccacaatctcatttgaggaatg	Fwd, 3' d/s flanking region of <i>L. reuteri</i> R2lc <i>araT</i>
oVPL1762	aactggacaattccatcgaggtc	Rev, 3' d/s flanking region of <i>L. reuteri</i> R2lc <i>araT</i>
oVPL1763	aaatggattcattgggtttgagcc	Fwd, for screening of Δ <i>araT</i> in <i>L. reuteri</i> R2lc
oVPL1758	aacgacggccagtgaaatcgagctcggaatcctgcccgtatgaattcgattgactgt	Rev, for screening of Δ <i>araT</i> in <i>L. reuteri</i> R2lc
oVPL1759	tcgttgctcataagcatcttcttcttgtagtagctggcatgataaattcctctatat	LCR bridging oligo for pORI- <i>ddIF258Y::araT</i> deletion cassette
oVPL1760	aagagcattacctcaaatgagattgtggcaatcctctagagctgacctgcaggcatgcaa	LCR bridging oligo for pORI- <i>ddIF258Y::araT</i> deletion cassette

oligo name	sequence (5'-3') [†]	Target/comment [#]
oVPL1555	agtcataaagccaacccaaaagg	Fwd, 5' u/s flanking region of <i>L. reuteri</i> DSM 17938 <i>sdpA</i>
oVPL1556	tgcttaaatgttaaaatgaaatagac	Rev, 5' u/s flanking region of <i>L. reuteri</i> DSM 17938 <i>sdpA</i>
oVPL1557	tgcataaaaaactcctctataatatac	Fwd, 3' u/s flanking region of <i>L. reuteri</i> DSM 17938 <i>sdpA</i>
oVPL1558	tcataccggcctgatcgg	Rev, 3' u/s flanking region of <i>L. reuteri</i> DSM 17938 <i>sdpA</i>
oVPL1559	aaacgacggccagtgaaatcgagctcggaagtcataaagccaacccaaaaggaattat	LCR bridging oligo for pORI- <i>ddlF258Y::sdpA</i> deletion cassette
oVPL1560	agtcatttcattttaaacgattaagcatgcataaaaaactcctctataatatacaat	LCR bridging oligo for pORI- <i>ddlF258Y::sdpA</i> deletion cassette
oVPL1561	gtaatatgcgaaccgatcaggccggatgaatcctctagagtcgacctgcaggcatgcaa	LCR bridging oligo for pORI- <i>ddlF258Y::sdpA</i> deletion cassette
oVPL2065	aaaaacaactaacaattatctaacagtaacc	Fwd, 5' u/s flanking region of <i>L. reuteri</i> DSM 17938 <i>sdpB</i>
oVPL2066	gaagactaattctagacaagtg	Rev, 5' u/s flanking region of <i>L. reuteri</i> DSM 17938 <i>sdpB</i>
oVPL2067	taacgttggaattccttattcattcc	Fwd, 3' u/s flanking region of <i>L. reuteri</i> DSM 17938 <i>sdpB</i>
oVPL2068	ttcatgtcatcgtaaatcaagg	Rev, 3' u/s flanking region of <i>L. reuteri</i> DSM 17938 <i>sdpB</i>
oVPL2069	aaacgacggccagtgaaatcgagctcggaaaaaacaactaacaattatctaacagtaac	LCR bridging oligo for pORI- <i>ddlF258Y::sdpB</i> deletion cassette
oVPL2070	taactattcactgtctagaattagcttctaacglttggaattccttattcattcctc	LCR bridging oligo for pORI- <i>ddlF258Y::sdpB</i> deletion cassette
oVPL2071	cgagttccttgaattacgcatgacatgaaatcctctagagtcgacctgcaggcatgcaa	LCR bridging oligo for pORI- <i>ddlF258Y::sdpB</i> deletion cassette
oVPL2072	aaccaccaatcccaagctatg	Fwd, for screening of Δ <i>sdpB</i> in <i>L. reuteri</i> DSM 17938
oVPL2073	tgcttatcaacaagtagattgcc	Rev, for screening of Δ <i>sdpB</i> in <i>L. reuteri</i> DSM 17938
oVPL2589	ctatcagttgccatcgatggcgacg	Fwd, amplifies promoter region of <i>L. plantarum</i> BAA-793 <i>Ddl</i>
oVPL2590	ttattccgcgtctaacgcaacgaaa	Rev, amplifies <i>L. plantarum</i> BAA-793 <i>Ddl</i>
oVPL2591	aaaataaataaggagcactcaccatgctatcagttgcatcgatggcgacgcagg	Bridging oligo for <i>L. plantarum</i> BAA-793 <i>Ddl</i> and pNZ8048 backbone.
oVPL2592	tatgatttcgttcggttagacgccaataatagttctagagctcaagcttctttgaa	Bridging oligo for <i>L. plantarum</i> BAA-793 <i>Ddl</i> and pNZ8048 backbone.
oVPL2593	/5phos/tcacaccactcgcacccagctacttattatagctagaacg	Rev, modifies F260Y of <i>ddl</i> of <i>L. plantarum</i> BAA-793
oVPL2594	/5phos/cgttcgaattaccagtcgaattacc	Fwd, amplifies the <i>ddl</i> of <i>L. plantarum</i> BAA-793
oVPL2694	taatcgttgtagctctcagcccca	Combined with oVPL2072-oVPL2073 for screening of Δ <i>sdpB</i> in <i>L. reuteri</i> DSM 17938
oVPL2841	aaacgacgatgcaatcacag	Fwd, amplifies GAPDH gene of <i>L. salivarius</i> CCUG 47825. For qPCR.
oVPL2842	cagcaccagttgagtgagga	Rev, amplifies GAPDH gene of <i>L. salivarius</i> CCUG 47825. For qPCR.
oVPL2845	cctgctcgtaaggctctc	Fwd, amplifies native <i>ddl</i> of <i>L. salivarius</i> CCUG 47825. For qPCR.
oVPL2846	acggattgcacctaacttg	Rev, amplifies native <i>ddl</i> of <i>L. salivarius</i> CCUG 47825. For qPCR.
oVPL2849	tggaatgctcgaatcttaggc	Fwd, amplifies <i>ddlF260Y</i> of pVPL3933. For qPCR.
oVPL2850	aaacgcgctgacgacatct	Rev, amplifies <i>ddlF260Y</i> of pVPL3933. For qPCR.

oVPL: Van Pijkeren Lab oligonucleotide identification number.

[†]: /5phos/: indicates oligonucleotides is phosphorylated on 5'-end;

[#]: LCR: ligase cycling reaction (8). Fwd: forward; Rev: reverse; GAPDH: glyceraldehyde-3-phosphate dehydrogenase (YP_536057.1). *pduCDE*: glycerol dehydratase large subunit PduC, glycerol dehydratase medium subunit PduD, glycerol dehydratase small subunit PduE (BAG26151, BAG26150.1, BAG26149.1); *araT*: aromatic amino acid aminotransferase (MG822655); *sdpA*: bifunctional phosphodiesterase (WP_003672341.1); *sdpB*: hypothetical protein and LPXTG-motif cell wall anchor domain protein (WP_013923860.1 , AEI56985.1); *acs1*: acyl-CoA synthetase (WP_014570113.1); The accession number listed can be found on <https://www.ncbi.nlm.nih.gov>.

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