Supplemental Table S1: Bacterial strains, vectors and primers used in this study.

L. reuteri Strains	Description	Source
WT 6475	*L. reuteri* ATCC PTA 6475, isolated from Finnish mother's milk	BioGaia AB (Stockholm, Sweden)
WT 6475_pJP042	*L. reuteri* ATCC PTA 6475_pJP042, Em^r, harboring the recombineering plasmid pJP042	(22)
6475::*rsiR* (HMPREF0536_10683)	*L. reuteri* ATCC PTA 6475 *rsiR*::pORI28, Em^r, *rsiR* insertion mutant	This study
6475*rsiR*-Stop	*L. reuteri* ATCC PTA 6475 *rsiR*::STOP, recombineered mutant with stop codon mutation in *rsiR*	This study
6475Δ*rsiR*	*L. reuteri* ATCC PTA 6475 **Δ***rsiR* , recombineered mutant with stop codon deletion in *rsiR*	This study
6475::*rsiR* _pJKS104	*L. reuteri* ATCC PTA 6475 *rsiR*::pORI28_pJKS104, Em^r, Cm^r, complemented *rsiR* insertion mutant	This study
WT 6475_pPH-R1	*L. reuteri* ATCC PTA 6475_pPH-R1, Cm^r, WT 6475 harboring the *gusA* reporter plasmid	This study
6475Δ*rsiR* _pPH-R1	*L. reuteri* ATCC PTA 6475 **Δ***rsiR*_pPH-R1, Cm^r, *rsiR* deletion mutant harboring the gusA reporter plasmid	This study

Vector	**Description**	**Source**
pVE6007	Cm^{r} *repA*-positive temperature-sensitive derivative of pWV01	(21)
pORI28	Em^{r} *repA*-negative derivative of pWV01	(20)
pJP059	Cm^{r} *plasmid used to evaluate transformation efficiency for recombineering*	(22)
pCR®2.1	Cloning vector	Invitrogen, Carlsbad, CA
pJKS100	*E. coli – L. reuteri* shuttle vector containing *L. lactis* P_{23} promoter, Cm^{r}	(24)
pJKS104	pJKS100 derivative expressing 6475 *rsiR* gene under control of its natural promoter, Cm^{r}	This study
pGK12	Contained hyperactive *gusA3* reporter gene	(30)
pPH-R1	pJKS100 derivative expressing *gusA* reporter gene from pGK12, under control of P_{hdcAB} promoter	This study
Recombineering oligonucleotide	**Sequence (5' to 3')**	**Gene targeted**
oJP577	TCAAACCACCAGGACCAAGCGCTGAAAGACGACGCTTTCTGCTTAATTCACCTAATGGGTTGGTTTGATCCATGAACTGG, positive control for recombineering	*rpoB* (22)

o977stop	CGGGTAGAATAAGACTTGAGGAGGTCTTTAGAATGAACCTATGATAACTTATTGTAGGAATTATTATGATTGTATTAGCC, for stop codon mutation	*rsiR*
oΔ977	CATCCGGGTAGAATAAGACTTGAGGAGGTCTTTAGAATGGAATAAATTATGCTAATTATTGGGCAATCCAATGATATTCGCTTTTAGC, for clean deletion mutant	*rsiR*
Primers	**Primer sequence (5’ to 3’)**	**Gene targeted**
rsiR-F	GCAAATCAGAAGATAATTTCTGGTTTGC, for *rsiR* cloning	*rsiR*
rsiR-R	CCAGCTAGCCCAATTAGTCTAAAACAG, for *rsiR* cloning	*rsiR*
cat-F	GGCATATCAAATGAACTTTAATAAAATTGA	*cat*
cat-R	CGGCATTATCTCATATTATAAAAGCCAG	*cat*
KJP15	GTTCTTGAATCTAATCAATATG, for screening of recombineered mutants	*rsiR*
KJP16	CCATTGAGCAATTTTAATAGTTG, for screening of recombineered mutants	*rsiR*
KJP33	GGTCTTTAGAATGAACCTATG	*rsiR*

P_{hdcAB}-F	TGACGGATCCTAACAGGAACTAATGGTAAGGGATC, amplification of P_{hdcAB} promoter	P_{hdcAB} promoter
P_{hdcAB}-R	TGACGAATTCCGCCTGTTTCCAATGTATTATCA, amplification of P_{hdcAB} promoter	P_{hdcAB} promoter
LR0977 5'-BHI	TGACGGATCCTAACGATTGTTAGAGGTTTTGGGAAT, amplification of pORI28 insert	*rsiR*
LR0977 3'-ERI	TGACGAATTCGCAATGGAAGATAGATAGTACAC, amplification of pORI28 insert	*rsiR*
qPCR Primer Name	**Primer Sequence**	**Universal Probe Number**
rpoB-F	CGTGATACTTCATTACGTGTTCCT	77
rpoB-R	AGTGAAGACTTTAACATCTTGGATGA	
hdcA-F	GCACTAACGATAACCGTCGTC	1
hdcA-R	CACCCTTATTAGCACAAACAATGA	
hdcB-F	TGCAAGATCGGATATCACAAA	162
hdcB-F	TTGACGAATTCAACCTTCTTTACA	
hdcP-F	TCCCTACGGATACCAAGCAC	15

hdcP-R	AGAGGAACGCTAAGACACCAAT	

Supplemental Table S2: RNA-seq data revealed 195 *L. reuteri* genes downregulated >1.5-fold in 6475*rsiR*-Stop mutant compared to WT 6475.

Locus tag	Protein ID	Gene name	Annotation	Fold downregulation
HMPREF0536_11827	ZP_08162905.1	*hdcA*	histidine decarboxylase subunit proenzyme	3.76
HMPREF0536_11825	ZP_08162903.1	*hisS*	histidine-tRNA ligase	3.70
HMPREF0536_10674	ZP_08161754.1	*fabD*	malonyl-CoA-[acyl-carrier-protein] transacylase	3.41
HMPREF0536_10758	ZP_08161837.1	*scrK2*	fructokinase	3.18
HMPREF0536_10672	ZP_08161752.1	*fabH*	3-oxoacyl-(acyl-carrier-protein) synthase III	3.05
HMPREF0536_11463	ZP_08162541.1	*rplV*	50S ribosomal protein L22	2.76
HMPREF0536_10052	ZP_08161134.1	*N/A*	GDSL family lipase	2.72
HMPREF0536_10148	ZP_08161229.1	*N/A*	SNF2/helicase domain protein	2.67

HMPREF0536_10757	ZP_08161836.1	*scrK*	fructokinase	2.65
HMPREF0536_10827	ZP_08161906.1	*N/A*	prophage Lp2 protein 43	2.62
HMPREF0536_10827	ZP_08161906.1	*N/A*	prophage Lp2 protein 43	2.62
HMPREF0536_10761	ZP_08161840.1	*lrgA*	murein hydrolase regulator LrgA	2.62
HMPREF0536_10036	ZP_08161118.1	*N/A*	phage portal protein	2.52
HMPREF0536_11982	ZP_08163060.1	*N/A*	hypothetical protein	2.51
HMPREF0536_10095	ZP_08161176.1	*lacZ*	beta-galactosidase 1	2.41
HMPREF0536_10759	ZP_08161838.1	*ldh*	L-lactate dehydrogenase	2.40
HMPREF0536_10141	ZP_08161222.1	*mmuM*	homocysteine S-methyltransferase	2.39
HMPREF0536_11780	ZP_08162858.1	*N/A*	ABC superfamily ATP binding cassette transporter	2.29
HMPREF0536_10760	ZP_08161839.1	*lrgB*	murein hydrolase regulator LrgB	2.28

HMPREF0536_10169	ZP_08161250.1	*N/A*	transposase	2.26
HMPREF0536_11485	ZP_08162563.1	*lysP2*	APC family lysine transporter	2.26
HMPREF0536_11864	ZP_08162942.1	*npp*	type I phosphodiesterase /nucleotide pyrophosphatase	2.25
HMPREF0536_11904	ZP_08162982.1	*N/A*	cell surface hydrolase	2.24
HMPREF0536_11817	ZP_08162895.1	*N/A*	MATE efflux family protein	2.21
HMPREF0536_10679	ZP_08161759.1	*accC*	acetyl-CoA carboxylase	2.20
HMPREF0536_10041	ZP_08161123.1	*N/A*	hypothetical protein	2.18
HMPREF0536_11567	ZP_08162645.1	*N/A*	phosphatase	2.14
HMPREF0536_11913	ZP_08162991.1	*N/A*	deaminase-reductase domain protein	2.13
HMPREF0536_10043	ZP_08161125.1	*N/A*	phage major tail protein	2.12
HMPREF0536_11876	ZP_08162954.1	*N/A*	nitroreductase	2.10
HMPREF0536_11621	ZP_08162699.1	*N/A*	YhgE/Pip domain	2.09

			protein	
HMPREF0536_11988	ZP_08163066.1	*ldhA3*	D-lactate dehydrogenase	2.09
HMPREF0536_10820	ZP_08161899.1	*N/A*	phage lysin	2.08
HMPREF0536_11939	ZP_08163017.1	*trmE*	tRNA modification GTPase TrmE	2.07
HMPREF0536_10046	ZP_08161128.1	*ppx*	exopolyphosphatase	2.05
HMPREF0536_10807	ZP_08161886.1	*gntK*	gluconokinase	2.04
HMPREF0536_10634	ZP_08161714.1	*N/A*	RNA-directed DNA polymerase	2.01
HMPREF0536_11610	ZP_08162688.1	*N/A*	hypothetical protein	2.01
HMPREF0536_10346	ZP_08161426.1	*aad*	aldehyde-alcohol dehydrogenase	2.01
HMPREF0536_11903	ZP_08162981.1	*mhpD*	2-oxopent-4-enoate hydratase	2.00
HMPREF0536_12008	ZP_08163086.1	*gshA*	glutamate-cysteine ligase	2.00
HMPREF0536_10783	ZP_08161862.1	*N/A*	major facilitator transporter	2.00
HMPREF0536_11970	ZP_08163048.1	*N/A*	alpha/beta knot family protein	1.98

HMPREF0536_11902	ZP_08162980.1	*hicD3*	L-2-hydroxyisocaproate dehydrogenase	1.97
HMPREF0536_11142	ZP_08162221.1	*plsX*	fatty acid/phospholipid synthesis protein PlsX	1.97
HMPREF0536_10105	ZP_08161186.1	*N/A*	hypothetical protein	1.95
HMPREF0536_10183	ZP_08161264.1	*nhaC*	NhaC family sodium:proton (Na+:H+) antiporter	1.95
HMPREF0536_11824	ZP_08162902.1	*nhaC2*	NhaC family sodium:proton (Na+:H) antiporter	1.94
HMPREF0536_12047	ZP_08163125.1	*glnP4*	glutamine ABC superfamily ATP binding cassette transporter	1.92
HMPREF0536_10107	ZP_08161188.1	*sdhA*	L-serine ammonia-lyase alpha subunit	1.92

HMPREF0536_10210	ZP_08161291.1	*metN*	methionine ABC superfamily ATP binding cassette transporter	1.91
HMPREF0536_11487	ZP_08162565.1	*N/A*	phosphatase	1.90
HMPREF0536_11462	ZP_08162540.1	*rpsC*	30S ribosomal protein S3	1.90
HMPREF0536_11085	ZP_08162164.1	*mraY*	phospho-N-acetylmuramoyl-pentapeptide-transferase	1.89
HMPREF0536_11735	ZP_08162813.1	*pduD*	propanediol dehydratase medium subunit	1.88
HMPREF0536_10049	ZP_08161131.1	*N/A*	chromosome segregation ATPase family protein	1.88
HMPREF0536_11723	ZP_08162801.1	*pduQ*	propanol dehydrogenase	1.88
HMPREF0536_10755	ZP_08161834.1	*aga*	alpha-galactosidase	1.88
HMPREF0536_10109	ZP_08161190.1	*trxB*	thioredoxin-	1.88

			disulfide reductase	
HMPREF0536_11488	ZP_08162566.1	*N/A*	hypothetical protein	1.87
HMPREF0536_11980	ZP_08163058.1	*macB*	macrolide export ABC superfamily ATP binding cassette transporter	1.86
HMPREF0536_11313	ZP_08162391.1	*N/A*	hypothetical protein	1.85
HMPREF0536_11526	ZP_08162604.1	*dacC*	D-alanyl-D-alanine serine-type carboxypeptidase	1.85
HMPREF0536_10268	ZP_08161349.1	*N/A*	RNA-directed DNA polymerase	1.85
HMPREF0536_10677	ZP_08161757.1	*accB*	acetyl-CoA carboxylase biotin carboxyl carrier subunit	1.85
HMPREF0536_10211	ZP_08161292.1	*metQ*	D-methionine ABC superfamily ATP binding cassette transporter	1.83

HMPREF0536_11529	ZP_08162607.1	*N/A*	MFS family major facilitator transporter	1.83
HMPREF0536_10764	ZP_08161843.1	*N/A*	band 7 family membrane protein	1.82
HMPREF0536_10128	ZP_08161209.1	*rfbD*	dTDP-4-dehydrorhamnose reductase	1.82
HMPREF0536_11742	ZP_08162820.1	*N/A*	hypothetical protein	1.80
HMPREF0536_11030	ZP_08162109.1	*comEA*	competence protein ComEA	1.79
HMPREF0536_11116	ZP_08162195.1	*N/A*	RecT protein	1.79
HMPREF0536_11553	ZP_08162631.1	*pstA*	phosphate ABC superfamily ATP binding cassette transporter	1.79
HMPREF0536_10316	ZP_08161396.1	*metC*	cystathionine beta-lyase	1.79
HMPREF0536_10675	ZP_08161755.1	*fabG2*	3-oxoacyl-[acyl-carrier-protein] reductase	1.78
HMPREF0536_11414	ZP_08162492.1	*N/A*	RNA-directed DNA	1.78

			polymerase	
HMPREF0536_11737	ZP_08162815.1	*pduB*	propanediol utilization protein PduB	1.77
HMPREF0536_11400	ZP_08162478.1	*N/A*	hypothetical protein	1.77
HMPREF0536_10822	ZP_08161901.1	*N/A*	prophage Lp2 protein 48	1.77
HMPREF0536_10217	ZP_08161298.1	*N/A*	aminotransferase	1.76
HMPREF0536_10475	ZP_08161555.1	*N/A*	hypothetical protein	1.76
HMPREF0536_11316	ZP_08162394.1	*N/A*	hypothetical protein	1.75
HMPREF0536_10033	ZP_08161115.1	*N/A*	hypothetical protein	1.75
HMPREF0536_11857	ZP_08162935.1	*tdh*	L-threonine 3-dehydrogenase	1.75
HMPREF0536_12022	ZP_08163100.1	*N/A*	glutamine amidotransferase class-I domain protein	1.75
HMPREF0536_11422	ZP_08162500.1	*rumA*	23S rRNA (uracil-5-)-	1.75

			methyltransferase	
HMPREF0536_10942	ZP_08162021.1	*glnP2*	glutamine ABC superfamily ATP binding cassette transporter	1.75
HMPREF0536_10301	ZP_08161381.1	*N/A*	phosphoesterase	1.75
HMPREF0536_10279	ZP_08161359.1	*N/A*	hydratase/decarboxylase	1.74
HMPREF0536_11938	ZP_08163016.1	*gidA*	glucose inhibited division protein A	1.74
HMPREF0536_10747	ZP_08161826.1	*dnaD*	DNA replication protein DnaD	1.74
HMPREF0536_11954	ZP_08163032.1	*rplI*	50S ribosomal protein L9	1.73
HMPREF0536_10603	ZP_08161683.1	*N/A*	hypothetical protein	1.72
HMPREF0536_12046	ZP_08163124.1	*serS*	serine--tRNA ligase	1.72
HMPREF0536_10146	ZP_08161227.1	*N/A*	hypothetical protein	1.72
HMPREF0536_10373	ZP_08161453.1	*N/A*	carbon-nitrogen family hydrolase	1.72
HMPREF0536_11451	ZP_08162529.1	*rpsE*	30S ribosomal	1.72

			protein S5	
HMPREF0536_12042	ZP_08163120.1	*N/A*	surface protein	1.71
HMPREF0536_12074	ZP_08163152.1	*pyrC*	dihydroorotase	1.71
HMPREF0536_10249	ZP_08161330.1	*pyrG*	CTP synthase	1.70
HMPREF0536_10838	ZP_08161917.1	*N/A*	hypothetical protein	1.70
HMPREF0536_11644	ZP_08162722.1	*N/A*	transposase	1.70
HMPREF0536_11518	ZP_08162596.1	*lysR*	malolactic regulator	1.70
HMPREF0536_11143	ZP_08162222.1	*recG*	DNA helicase RecG	1.69
HMPREF0536_10302	ZP_08161382.1	*N/A*	hypothetical protein	1.69
HMPREF0536_11901	ZP_08162979.1	*N/A*	aminotransferase	1.69
HMPREF0536_11730	ZP_08162808.1	*N/A*	carbon dioxide concentrating mechanism/carboxysome shell protein	1.69
HMPREF0536_11734	ZP_08162812.1	*dhaE*	glycerol dehydratase	1.68
HMPREF0536_11804	ZP_08162882.1	*N/A*	hypothetical protein	1.68

HMPREF0536_10470	ZP_08161550.1	*N/A*	hypothetical protein	1.68
HMPREF0536_10038	ZP_08161120.1	*N/A*	phage major head protein	1.68
HMPREF0536_10763	ZP_08161842.1	*lytS*	sensor histidine kinase LytS	1.67
HMPREF0536_11577	ZP_08162655.1	*adhB*	alcohol dehydrogenase	1.67
HMPREF0536_11568	ZP_08162646.1	*N/A*	hypothetical protein	1.67
HMPREF0536_10635	ZP_08161715.1	*N/A*	hypothetical protein	1.67
HMPREF0536_10375	ZP_08161455.1	*panE*	2-dehydropantoate 2-reductase	1.67
HMPREF0536_10035	ZP_08161117.1	*N/A*	phage terminase large subunit	1.66
HMPREF0536_11917	ZP_08162995.1	*N/A*	hypothetical protein	1.66
HMPREF0536_11863	ZP_08162941.1	*N/A*	hypothetical protein	1.66
HMPREF0536_10472	ZP_08161552.1	*nhaP*	Na-H antiporter	1.66
HMPREF0536_11729	ZP_08162807.1	*pduL*	propanediol utilization protein	1.65

			PduL	
HMPREF0536_11571	ZP_08162649.1	*N/A*	transposase	1.65
HMPREF0536_11733	ZP_08162811.1	*dhaF*	glycerol dehydratase reactivation factor large subunit	1.65
HMPREF0536_10859	ZP_08161938.1	*N/A*	hypothetical protein	1.65
HMPREF0536_10957	ZP_08162036.1	*lexA*	LexA repressor	1.65
HMPREF0536_11504	ZP_08162582.1	*lmrB2*	MFS family major facilitator transporter	1.64
HMPREF0536_11061	ZP_08162140.1	*lysA*	diaminopimelate decarboxylase	1.64
HMPREF0536_11736	ZP_08162814.1	*dhaB*	glycerol dehydratase	1.64
HMPREF0536_11966	ZP_08163044.1	*vicX*	metallo-beta-lactamase superfamily protein	1.64
HMPREF0536_10222	ZP_08161303.1	*trpS*	tryptophan--tRNA ligase	1.64
HMPREF0536_12005	ZP_08163083.1	*dltr*	DNA-binding response regulator	1.64

HMPREF0536_11753	ZP_08162831.1	*N/A*	MFS family major facilitator transporter	1.64
HMPREF0536_12033	ZP_08163111.1	*pyraA2*	carbamoyl-phosphate synthase	1.63
HMPREF0536_10393	ZP_08161473.1	*N/A*	MaoC family protein	1.63
HMPREF0536_10236	ZP_08161317.1	*N/A*	zinc/iron ABC superfamily ATP binding cassette transporter	1.63
HMPREF0536_10037	ZP_08161119.1	*clpP*	ATP-dependent Clp protease	1.63
HMPREF0536_11802	ZP_08162880.1	*brnQ2*	LIVCS family branched chain amino acid:cation symporter	1.62
HMPREF0536_10503	ZP_08161583.1	*N/A*	multitransmembrane protein	1.62
HMPREF0536_10818	ZP_08161897.1	*N/A*	hypothetical protein	1.62
HMPREF0536_10455	ZP_08161535.1	*N/A*	DMT superfamily	1.62

			drug/metabolite transporter	
HMPREF0536_11692	ZP_08162770.1	*hemA*	glutamyl-tRNA reductase	1.61
HMPREF0536_11271	ZP_08162349.1	*dgk*	deoxynucleoside kinase	1.61
HMPREF0536_10722	ZP_08161801.1	*N/A*	LysR family transcriptional regulator	1.61
HMPREF0536_11055	ZP_08162134.1	*asd*	aspartate-semialdehyde dehydrogenase	1.61
HMPREF0536_11506	ZP_08162584.1	*rluD3*	ribosomal large subunit pseudouridine synthase D	1.61
HMPREF0536_11493	ZP_08162571.1	*N/A*	protein of hypothetical function DUF125	1.61
HMPREF0536_11732	ZP_08162810.1	*N/A*	propanediol dehydratase reactivation protein PduH	1.60

HMPREF0536_10986	ZP_08162065.1	*tsf*	elongation factor EF1B	1.60
HMPREF0536_11611	ZP_08162689.1	*nox*	NADH oxidase	1.60
HMPREF0536_11551	ZP_08162629.1	*phoU*	phosphate transport system regulatory protein PhoU	1.60
HMPREF0536_11973	ZP_08163051.1	*dhaT*	N/A	1.60
HMPREF0536_10824	ZP_08161903.1	*N/A*	hypothetical protein	1.60
HMPREF0536_11051	ZP_08162130.1	*glxK*	glycerate kinase	1.60
HMPREF0536_11554	ZP_08162632.1	*pstC*	phosphate ABC superfamily ATP binding cassette transporter	1.60
HMPREF0536_10259	ZP_08161340.1	*N/A*	phosphoglycerate mutase	1.59
HMPREF0536_10319	ZP_08161399.1	*fliY*	amino acid ABC superfamily ATP binding cassette transporter	1.59
HMPREF0536_10413	ZP_08161493.1	*N/A*	APC family amino acid transporter	1.57

HMPREF0536_10094	ZP_08161175.1	*lacS*	GPH family lactose:cation symporter	1.57
HMPREF0536_10732	ZP_08161811.1	*N/A*	DegV family protein	1.57
HMPREF0536_10561	ZP_08161641.1	*glnQ*	glutamine ABC superfamily ATP binding cassette transporter	1.56
HMPREF0536_11066	ZP_08162145.1	*N/A*	tetratricopeptide TPR_2 repeat protein	1.56
HMPREF0536_12003	ZP_08163081.1	*N/A*	hypothetical protein	1.56
HMPREF0536_10725	ZP_08161804.1	*N/A*	glycerol-3-phosphate dehydrogenase	1.56
HMPREF0536_10652	ZP_08161732.1	*mobB*	molybdopterin-guanine dinucleotide biosynthesis protein MobB	1.56
HMPREF0536_12031	ZP_08163109.1	*N/A*	major facilitator	1.55

			transporter	
HMPREF0536_11961	ZP_08163039.1	*N/A*	multidrug ABC superfamily ATP binding cassette transporter	1.55
HMPREF0536_10527	ZP_08161607.1	*araA*	L-arabinose isomerase	1.55
HMPREF0536_10247	ZP_08161328.1	*N/A*	hypothetical protein	1.55
HMPREF0536_12028	ZP_08163106.1	*tagE2*	poly(glycerol-phosphate) alpha-glucosyltransferase	1.55
HMPREF0536_11255	ZP_08162333.1	*ldh3*	L-lactate dehydrogenase	1.55
HMPREF0536_10762	ZP_08161841.1	*lytR*	response regulator	1.54
HMPREF0536_10819	ZP_08161898.1	*N/A*	ChvD family ATP-binding cassette protein	1.53
HMPREF0536_11539	ZP_08162617.1	*cysK*	cysteine synthase	1.53
HMPREF0536_10253	ZP_08161334.1	*N/A*	PTS family porter cellobiose-specific component IIC	1.53

HMPREF0536_11715	ZP_08162793.1	*N/A*	NADPH-dependent FMN reductase	1.53
HMPREF0536_11465	ZP_08162543.1	*rplB*	50S ribosomal protein L2	1.53
HMPREF0536_10531	ZP_08161611.1	*N/A*	universal stress protein	1.53
HMPREF0536_11873	ZP_08162951.1	*N/A*	GNAT family acetyltransferase	1.53
HMPREF0536_11448	ZP_08162526.1	*secY*	preprotein translocase subunit SecY	1.53
HMPREF0536_11790	ZP_08162868.1	*N/A*	hypothetical protein	1.52
HMPREF0536_11783	ZP_08162861.1	*cysK2*	cysteine synthase A	1.52
HMPREF0536_10119	ZP_08161200.1	*N/A*	sigma-70 family RNA polymerase sigma factor	1.52
HMPREF0536_10504	ZP_08161584.1	*pyrP*	NCS2 family uracil:cation symporter-2	1.52
HMPREF0536_10756	ZP_08161835.1	*N/A*	LacI family	1.52

			transcriptional regulator	
HMPREF0536_10816	ZP_08161895.1	*N/A*	GDSL family lipase	1.52
HMPREF0536_11042	ZP_08162121.1	*pdhA*	pyruvate dehydrogenase complex E1 component alpha subunit	1.52
HMPREF0536_10556	ZP_08161636.1	*radC*	DNA repair protein RadC	1.52
HMPREF0536_10390	ZP_08161470.1	*comFC*	competence protein FC	1.51
HMPREF0536_10710	ZP_08161789.1	*uvrA2*	excision endonuclease subunit UvrA	1.51
HMPREF0536_11680	ZP_08162758.1	*N/A*	multidrug resistance ABC superfamily ATP binding cassette transporter	1.51
HMPREF0536_10776	ZP_08161855.1	*N/A*	hypothetical protein	1.51

HMPREF0536_11738	ZP_08162816.1	*N/A*	carbon dioxide concentrating mechanism/carboxysome shell protein	1.51
HMPREF0536_10214	ZP_08161295.1	*N/A*	fumarate reductase	1.51
HMPREF0536_11272	ZP_08162350.1	*N/A*	mannosyl-glycoprotein endo-beta-N-acetylglucosamidase	1.51
HMPREF0536_11059	ZP_08162138.1	*N/A*	M20/M25/M40 family peptidase	1.50

Supplemental Table S3: RNA-seq data revealed 143 *L. reuteri* genes upregulated >1.5-fold in 6475*rsiR*-Stop mutant compared to WT 6475.

Locus tag	Protein ID	Gene name	Annotation	Fold upregulation
HMPREF0536_10683	ZP_08161763.1	*N/A*	conserved hypothetical protein (*rsiR*)	29.57
HMPREF0536_10227	ZP_08161308.1	*N/A*	hypothetical protein	6.78
HMPREF0536_10417	ZP_08161497.1	*N/A*	hypothetical protein	4.80
HMPREF0536_11519	ZP_08162597.1	*N/A*	peptidyl-prolyl cis-trans isomerase	4.63
HMPREF0536_11799	ZP_08162877.1	*N/A*	hypothetical protein	4.27
HMPREF0536_10349	ZP_08161429.1	*nrdH*	glutaredoxin	4.13
HMPREF0536_11893	ZP_08162971.1	*N/A*	HesB/YadR/YfhF-family protein	3.75
HMPREF0536_10492	ZP_08161572.1	*N/A*	hypothetical protein	3.33
HMPREF0536_10136	ZP_08161217.1	*N/A*	transposase	3.16

HMPREF0536_11350	ZP_08162428.1	*N/A*	muramidase	3.14
HMPREF0536_11287	ZP_08162365.1	*N/A*	hypothetical protein	3.06
HMPREF0536_11028	ZP_08162107.1	*comEC*	ComE operon protein 3	2.86
HMPREF0536_11044	ZP_08162123.1	*N/A*	hypothetical protein	2.86
HMPREF0536_10880	ZP_08161959.1	*N/A*	hypothetical protein	2.85
HMPREF0536_10429	ZP_08161509.1	*secG*	preprotein translocase subunit SecG	2.78
HMPREF0536_11383	ZP_08162461.1	*N/A*	acetyltransferase	2.70
HMPREF0536_10471	ZP_08161551.1	*N/A*	hypothetical protein	2.64
HMPREF0536_11900	ZP_08162978.1	*N/A*	hypothetical protein	2.64
HMPREF0536_11082	ZP_08162161.1	*ftsQ*	cell division protein FtsQ	2.58
HMPREF0536_11920	ZP_08162998.1	*N/A*	YbaK/prolyl-tRNA synthetase associated region	2.50

HMPREF0536_10765	ZP_08161844.1	*N/A*	NOL1/NOP2/sun family protein	2.44
HMPREF0536_10621	ZP_08161701.1	*N/A*	hypothetical protein	2.39
HMPREF0536_10733	ZP_08161812.1	*fbpA*	fibronectin-binding protein	2.39
HMPREF0536_11559	ZP_08162637.1	*N/A*	hypothetical protein	2.34
HMPREF0536_10643	ZP_08161723.1	*moaD*	molybdenum cofactor biosynthesis protein small subunit	2.32
HMPREF0536_11588	ZP_08162666.1	*wecD*	acetyltransferase	2.28
HMPREF0536_11141	ZP_08162220.1	*acpA*	acyl carrier protein	2.27
HMPREF0536_10626	ZP_08161706.1	*N/A*	CRP/FNR family transcriptional regulator	2.26
HMPREF0536_10881	ZP_08161960.1	*N/A*	hypothetical protein	2.22
HMPREF0536_10579	ZP_08161659.1	*N/A*	protein of hypothetical	2.20

			function DUF965	
HMPREF0536_10877	ZP_08161956.1	*asp*	stress response regulator Gls24	2.19
HMPREF0536_11596	ZP_08162674.1	*N/A*	phage integrase family site-specific recombinase	2.18
HMPREF0536_11393	ZP_08162471.1	*N/A*	GNAT family acetyltransferase	2.17
HMPREF0536_11343	ZP_08162421.1	*N/A*	ISChy4 transposase	2.12
HMPREF0536_10622	ZP_08161702.1	*N/A*	hypothetical protein	2.11
HMPREF0536_10418	ZP_08161498.1	*N/A*	hypothetical protein	2.11
HMPREF0536_10575	ZP_08161655.1	*yajC*	translocase subunit	2.06
HMPREF0536_11140	ZP_08162219.1	*rnc2*	ribonuclease III	2.06
HMPREF0536_10903	ZP_08161982.1	*N/A*	tetratricopeptide repeat family protein	2.01
HMPREF0536_11418	ZP_08162496.1	*N/A*	phage integrase family	1.99

			integrase/recombinase	
HMPREF0536_10246	ZP_08161327.1	*N/A*	transposase	1.98
HMPREF0536_11375	ZP_08162453.1	*N/A*	hypothetical protein	1.97
HMPREF0536_12080	ZP_08163158.1	*N/A*	permease	1.97
HMPREF0536_11018	ZP_08162097.1	*mazG*	MazG nucleotide pyrophosphohydrolase	1.96
HMPREF0536_11831	ZP_08162909.1	*N/A*	arginine/ornithine APC family amino acid-polyamine-organocation transporter	1.96
HMPREF0536_10283	ZP_08161363.1	*hslR*	S4 domain protein	1.95
HMPREF0536_10991	ZP_08162070.1	*plsC*	1-acylglycerol-3-phosphate O-acyltransferase	1.94
HMPREF0536_11178	ZP_08162257.1	*N/A*	hypothetical protein	1.92
HMPREF0536_11830	ZP_08162908.1	*N/A*	hypothetical	1.91

			protein	
HMPREF0536_11157	ZP_08162236.1	*gmk*	guanylate kinase	1.89
HMPREF0536_11392	ZP_08162470.1	*N/A*	hypothetical protein	1.88
HMPREF0536_10348	ZP_08161428.1	*nrdE*	ribonucleotide-diphosphate reductase subunit alpha	1.86
HMPREF0536_10535	ZP_08161615.1	*N/A*	hypothetical protein	1.85
HMPREF0536_11480	ZP_08162558.1	*ctsR*	transcriptional regulator CtsR	1.84
HMPREF0536_10410	ZP_08161490.1	*N/A*	hypothetical protein	1.83
HMPREF0536_10911	ZP_08161990.1	*N/A*	membrane protein	1.83
HMPREF0536_10609	ZP_08161689.1	*N/A*	hypothetical protein	1.82
HMPREF0536_11078	ZP_08162157.1	*N/A*	cell division membrane protein	1.80
HMPREF0536_10684	ZP_08161764.1	*frnE*	protein-disulfide isomerase	1.78

HMPREF0536_11943	ZP_08163021.1	*rpmH*	50S ribosomal protein L34	1.78
HMPREF0536_11208	ZP_08162287.1	*N/A*	Na+ driven multidrug efflux pump	1.77
HMPREF0536_10995	ZP_08162074.1	*mvaS*	hydroxymethylglutaryl-CoA synthase	1.77
HMPREF0536_11859	ZP_08162937.1	*N/A*	hypothetical protein	1.77
HMPREF0536_10171	ZP_08161252.1	*N/A*	MutT/nudix family hydrolase	1.75
HMPREF0536_10367	ZP_08161447.1	*N/A*	M22 family O-sialoglycoprotein endopeptidase	1.74
HMPREF0536_10865	ZP_08161944.1	*N/A*	XRE family transcriptional regulator	1.74
HMPREF0536_11706	ZP_08162784.1	*cbiT*	precorrin-8W decarboxylase	1.73
HMPREF0536_10325	ZP_08161405.1	*N/A*	PIN domain protein	1.71
HMPREF0536_11762	ZP_08162840.1	*N/A*	IS30 family	1.71

			transposase	
HMPREF0536_10096	ZP_08161177.1	*N/A*	polyprenyl synthetase	1.70
HMPREF0536_10932	ZP_08162011.1	*dgkA*	diacylglycerol kinase	1.70
HMPREF0536_11033	ZP_08162112.1	*rsmD*	ribosomal RNA small subunit methyltransferase D	1.69
HMPREF0536_11347	ZP_08162425.1	*N/A*	hypothetical protein	1.69
HMPREF0536_11220	ZP_08162299.1	*N/A*	GTP-binding protein	1.67
HMPREF0536_10430	ZP_08161510.1	*N/A*	carboxylesterase	1.67
HMPREF0536_11008	ZP_08162087.1	*N/A*	phage/plasmid primase	1.67
HMPREF0536_11624	ZP_08162702.1	*N/A*	hypothetical protein	1.66
HMPREF0536_10462	ZP_08161542.1	*N/A*	response regulator	1.66
HMPREF0536_11419	ZP_08162497.1	*N/A*	type I restriction-modification system S subunit	1.65

HMPREF0536_11086	ZP_08162165.1	*pbp1*	penicillin-binding protein 1	1.65
HMPREF0536_11883	ZP_08162961.1	*N/A*	hypothetical protein	1.65
HMPREF0536_10280	ZP_08161360.1	*N/A*	CBS domain protein	1.65
HMPREF0536_10931	ZP_08162010.1	*era*	GTP-binding protein Era	1.65
HMPREF0536_11050	ZP_08162129.1	*N/A*	hypothetical protein	1.65
HMPREF0536_10347	ZP_08161427.1	*nrdF*	ribonucleotide-diphosphate reductase subunit beta	1.65
HMPREF0536_11183	ZP_08162262.1	*araT2*	aromatic amino acid aminotransferase	1.64
HMPREF0536_11599	ZP_08162677.1	*N/A*	hypothetical protein	1.63
HMPREF0536_10619	ZP_08161699.1	*thiD*	phosphomethylpyrimidine kinase	1.63
HMPREF0536_11171	ZP_08162250.1	*rpmA*	50S ribosomal	1.63

			protein L27	
HMPREF0536_11718	ZP_08162796.1	*N/A*	YjbE family integral membrane protein	1.62
HMPREF0536_11308	ZP_08162386.1	*ptsH*	PTS family porter component HPr	1.62
HMPREF0536_10558	ZP_08161638.1	*mreC*	rod shape-determining protein MreC	1.62
HMPREF0536_10442	ZP_08161522.1	*rbsD*	ribose ABC superfamily ATP binding cassette transporter	1.62
HMPREF0536_10572	ZP_08161652.1	*ruvA*	crossover junction ATP-dependent DNA helicase RuvA	1.62
HMPREF0536_10980	ZP_08162059.1	*cdsA*	phosphatidate cytidylyltransferase	1.61
HMPREF0536_11283	ZP_08162361.1	*fur*	fur family	1.61

			transcriptional regulator	
HMPREF0536_10856	ZP_08161935.1	*N/A*	RecT protein	1.61
HMPREF0536_10701	ZP_08161781.1	*N/A*	hypothetical protein	1.61
HMPREF0536_11063	ZP_08162142.1	*dapF*	diaminopimelate epimerase	1.61
HMPREF0536_11186	ZP_08162265.1	*miaA*	tRNA delta(2)-isopentenylpyrophosphate transferase	1.61
HMPREF0536_11626	ZP_08162704.1	*fur2*	fur family transcriptional regulator	1.61
HMPREF0536_11344	ZP_08162422.1	*N/A*	transposase	1.60
HMPREF0536_10908	ZP_08161987.1	*N/A*	hypothetical protein	1.60
HMPREF0536_10521	ZP_08161601.1	*ddlA*	D-alanine-D-alanine ligase	1.59
HMPREF0536_10468	ZP_08161548.1	*N/A*	cupredoxin family domain protein	1.59
HMPREF0536_11153	ZP_08162232.1	*fmt*	methionyl-tRNA	1.59

			formyltransferase	
HMPREF0536_11833	ZP_08162911.1	*N/A*	hypothetical protein	1.58
HMPREF0536_11285	ZP_08162363.1	*N/A*	VanZ like family protein	1.58
HMPREF0536_10108	ZP_08161189.1	*ahpC*	alkyl hydroperoxide reductase C	1.57
HMPREF0536_10252	ZP_08161333.1	*rpmE*	50S ribosomal protein L31	1.56
HMPREF0536_11987	ZP_08163065.1	*N/A*	OsmC family protein	1.56
HMPREF0536_10193	ZP_08161274.1	*N/A*	pyridine nucleotide-disulfide oxidoreductase	1.55
HMPREF0536_10103	ZP_08161184.1	*rpsN*	30S ribosomal protein S14	1.55
HMPREF0536_10659	ZP_08161739.1	*narI*	respiratory nitrate reductase	1.55
HMPREF0536_11944	ZP_08163022.1	*dnaA*	DNA-directed DNA replication	1.55

			initiator protein DnaA	
HMPREF0536_10704	ZP_08161783.1	*endA*	DNA-entry nuclease	1.54
HMPREF0536_10443	ZP_08161523.1	*fucP*	fucose permease	1.54
HMPREF0536_10382	ZP_08161462.1	*N/A*	Ala-tRNA(Pro) hydrolase	1.54
HMPREF0536_11881	ZP_08162959.1	*N/A*	oxidoreductase	1.53
HMPREF0536_11394	ZP_08162472.1	*gor2*	glutathione-disulfide reductase	1.53
HMPREF0536_10744	ZP_08161823.1	*recU*	recombination protein U	1.53
HMPREF0536_11007	ZP_08162086.1	*N/A*	phage head-tail adaptor	1.53
HMPREF0536_10522	ZP_08161602.1	*N/A*	hypothetical protein	1.53
HMPREF0536_10158	ZP_08161239.1	*N/A*	phosphoglycerate mutase	1.53
HMPREF0536_11346	ZP_08162424.1	*N/A*	hypothetical protein	1.53
HMPREF0536_11958	ZP_08163036.1	*N/A*	protein of hypothetical	1.53

			function DUF344	
HMPREF0536_10978	ZP_08162057.1	*proS*	proline--tRNA ligase	1.52
HMPREF0536_10610	ZP_08161690.1	*dedA*	DedA family membrane protein	1.52
HMPREF0536_10601	ZP_08161681.1	*N/A*	hypothetical protein	1.52
HMPREF0536_10385	ZP_08161465.1	*N/A*	hypothetical protein	1.52
HMPREF0536_11770	ZP_08162848.1	*N/A*	hypothetical protein	1.52
HMPREF0536_11787	ZP_08162865.1	*N/A*	XRE family transcriptional regulator	1.52
HMPREF0536_11326	ZP_08162404.1	*N/A*	transposase	1.52
HMPREF0536_11378	ZP_08162456.1	*N/A*	glutamate-cysteine ligase	1.52
HMPREF0536_11379	ZP_08162457.1	*N/A*	hypothetical protein	1.52
HMPREF0536_11360	ZP_08162438.1	*cps1D*	capsular biosynthesis protein	1.52

HMPREF0536_11348	ZP_08162426.1	*N/A*	putative autolysin	1.51
HMPREF0536_10100	ZP_08161181.1	*menG*	2-heptaprenyl-1	1.51
HMPREF0536_11198	ZP_08162277.1	*udk*	uridine kinase	1.51
HMPREF0536_10895	ZP_08161974.1	*N/A*	hypothetical protein	1.51
HMPREF0536_10449	ZP_08161529.1	*N/A*	acyltransferase	1.50
HMPREF0536_11945	ZP_08163023.1	*dnaN*	DNA-directed DNA polymerase III beta subunit	1.50
HMPREF0536_10737	ZP_08161816.1	*lspA*	signal peptidase II	1.50

Supplemental Table S4: Functional clustering analysis* of genes downregulated >1.5-fold in 6475*rsiR*-Stop mutant compared to WT 6475.

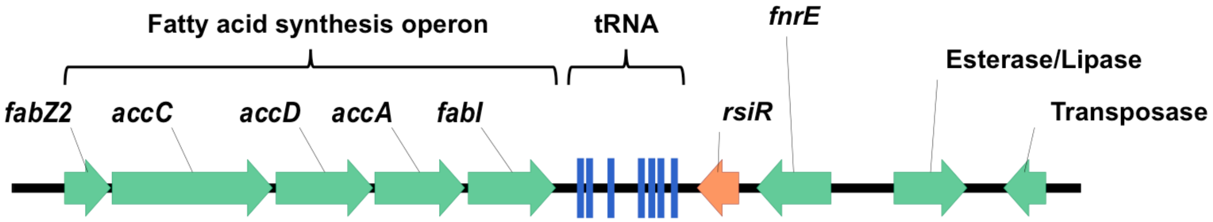
Annotation Cluster	**KEGG ID**	**P-value**	**Clustered Genes**
Cysteine and methionine metabolism	lrf00270	6.47E-04	*metC, asd, ldh, sdaAA, ldh3, cysK*
Fatty acid biosynthesis	lrf00061	9.89E-04	*accC, accB, fabG2, fabD, fabHA*
Glycerolipid metabolism	lrf00561	0.004	*glxK, dhaE/pduE, pduD, dhaB/pduC*
Pyruvate metabolism	lrf00620	0.005	*pdxB, aad, ldh, accC, accB, ldh3*
Propanoate metabolism	lrf00640	0.015	*ldh, accC, accB, ldh3*
Tyrosine metabolism	lrf00350	0.019	HMPREF0536_10279*, aad, mhpD*
Glycine, serine and threonine metabolism	lrf00260	0.037	*asd, glxK, sdaAA*
Two-component system	lrf02020	0.052	*lyts, lytR, lrgA, lrgB*

*RNA-seq data were analyzed using the complete *L. reuteri* JCM1112 reference genome. Genes downregulated >1.5-fold were subjected to DAVID functional clustering analysis with medium classification stringency to identify enriched biological themes according to KEGG Pathways. P-values were calculated using one-tailed Fisher Exact Test.

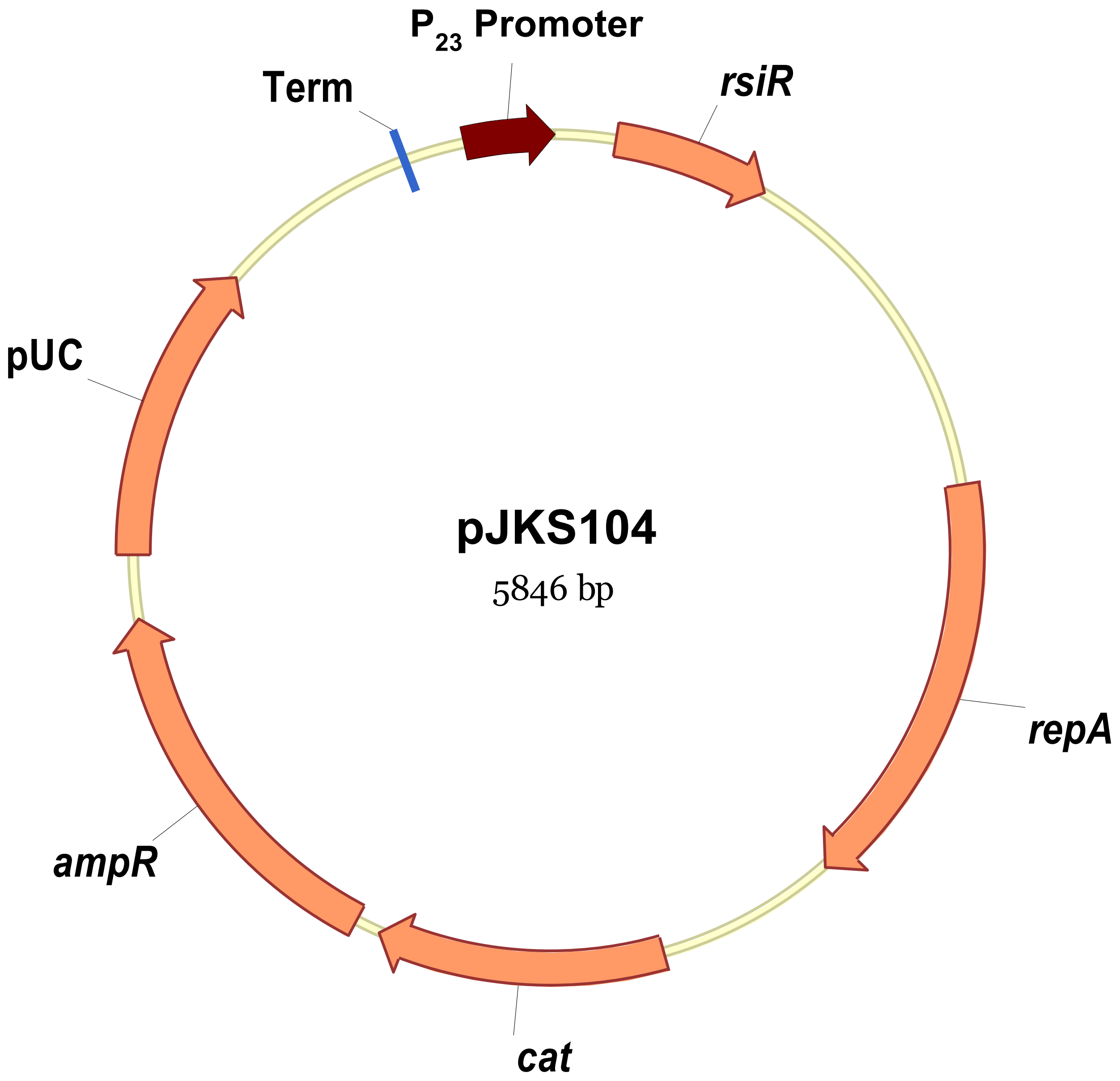
Supplemental Table S5: Differentially expressed genes in 6475*rsiR*-Stop mutant compared to WT 6475 that contain seeding sequence complementarity of 10 base pairs or longer.

Locus tag	Predicted gene product	Fold regulation compared to WT 6475
HMPREF0536_10932	Diacylglycerol kinase	1.70-fold upregulated
HMPREF0536_11833	Hypothetical protein	1.58-fold upregulated
HMPREF0536_10556	DNA repair protein RadC	1.53-fold upregulated
HMPREF0536_10449	Acyltransferase	1.50-fold upregulated
HMPREF0536_10758	Fructokinase	3.18-fold downregulated
HMPREF0536_10046	Exopolyphosphate	2.05-fold downregulated
HMPREF0536_11723	Propanol dehydrogenase *pduQ*	1.88-fold downregulated
HMPREF0536_10279	Hydratase/decarboxylase	1.74-fold downregulated

Supplemental Figure S1: Position of *rsiR* (HMPREF0536_10683) in the *L. reuteri* ATCC PTA 6475 chromosome. *fabZ2* = 3-hydroxyacyl-[acyl-carrier-protein] dehydratase gene **;** *accA* = Acetyl-coA carboxylase carboxyl transferase subunit alpha gene ; *accC* = Acetyl-CoA carboxylase, biotin carboxylase gene ; *accD* = Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta gene ; *fabl* = Enoyl-[acyl-carrier-protein] reductase [NADH] gene ; = *; fnrE* = predicted dithio-disulfide isomerase gene ; *rsiR* = *Lactobacillus reuteri*-Specific Immunoregulatory gene.



Supplemental Figure S2: The *L. reuteri*::*rsiR* Complementation vector, pJKS104. The *E. coli*-*L. reuteri* shuttle vector, pJKS104, contains a full-length copy of wild type *rsiR* under the control of P_{23} constitutive promoter. *ampR* = ampicilin resistance gene; *cat* = chloramphenicol resistance gene; pUC = *E. coli* origin of replication; *repA* = *L. reuteri* rolling circle replication protein; *rsiR* = *Lactobacillus reuteri*-Specific Immunoregulatory gene; Term = transcriptional terminator.



Supplemental Figure S3: Promoter activity of P_{hdcAB} was tested using the *gusA3* reporter gene in pPH-R1. *ampR* = ampicilin resistance gene; *cat* = chloramphenicol resistance gene; *gusA3* = hyperactive β-glucuronidase gene; P_{hdcAB} = putative *hdcAB* promoter region; pUC = *E. coli* origin of replication; *repA* = *L. reuteri* rolling circle replication protein; Term = transcriptional terminator.