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Coláiste na hOllscoile Corcaigh

Transcriptional control of central carbon metabolic flux in Bifidobacteria by two functionally similar, yet distinct LacI-type regulators

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Supplemental Tables

Supplemental Table S1. Sites found using PWM From Khoroshkin et al, 2016 40

Sites found using PWM from Khoroshkin et al., 2016							
Locus_tag	Gene/operon	Site	Pos*	Score	Binding AraQ	Binding MalR1	Additional information
Bbr_0032	malR5	aAaTGatAGCGCTtACAtTg	-264	4.59	+	+	AraQ regulon in Bifidobacteriaceae
		aAccGTGACCGCTaACAacg	-158	4.93			Also predicted as MalR5 sites
Bbr_0033	malE2	cgTTGTtAGCGGTCACggTt	-226	4.93			
		cAaTGTaAGCGGTatCAtTt	-120	4.59			
Bbr_0037	icfA	gATTGTGAGCGtTcTcAtTA	-115	5.13	NM	NM	
Bbr_0038	ahpC	TAaTGaGaaCGCTCACAATc	-173	5.13			
Bbr_0221	bfeU - bfeO	ggaTGTtAcCGCTCACAtgc	-221	4.82	NM	NM	
Bbr_0411	araQ	cAaTGTGAGCGGTCACgcgA	-112	5.38	+++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_0603	ctr	acaTgcGAGCGtTCACgATg	-101	4.56	NM	NM	
Bbr_0725	eno	cATTGTGAGCGGTCACAtcA	-110	5.99	+++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_0746	glgB - Bbr_745 - Bbr_744	cAaTGaGAGCGCTCACAATc	-108	5.3	+++	++	AraQ regulon in Bifidobacteriaceae
Bbr_0747	carD - ispF	gATTGTGAGCGGTcTcAtTg	-91	5.3			
Bbr_0757	pyk	cAgTGTGAGCGCTCACAaCg	-130	5.92	+++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_0772	pta - ackA	cAccGTGaaCGaTaACAacg	-76	4.59	NM	NM	AraQ regulon in Bifidobacteriaceae
Bbr_0787	pf1B - pf1A	gATTGgtAGCGCTCACAgAa	-135	5.27	++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_1003	tkt - tal	aATTGTGaaCGCTaACAgaA	-156	5.68	++	++	AraQ regulon in Bifidobacteriaceae
Bbr_1233	gap	cAaTGTGAGCGCTCACAaaA	-156	6.02	+++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_1237	galM	gATTGcGAGCGgTaACAATA	-46	4.74	NM	NM	AraQ regulon in Bifidobacteriaceae
Bbr_1273	ldh2	ggcTGTGAGCGCTaACAaAct	-160	5.77	+++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_1316	Bbr_1316	ctcTGTtAtCGtTCACAATg	-250	4.89	NM	NM	
Bbr_1419	rbsA1 - rbsC1 - rbsB1 - rbsD1 - rbsK2	gATTGTtAaCGTCTcCgAcA	-184	4.76	-	++	
Bbr_1650	malQ2	ccaTGTGAGCGGTcTcCAtgt	-138	4.51	NM	NM	AraQ regulon in Bifidobacteriaceae
Bbr_1685	Bbr_1685	ctATGTGaaCGCTcTcCAAgc	-232	4.75	NM	NM	
Bbr_1723	birA	acgTGTGAGCGtTCACAaccg	-107	5.2	NM	NM	AraQ regulon in Bifidobacteriaceae
Bbr_1724	Bbr_1724 - Bbr_1725	cggTGTGaaCGCTCACAcgt	-158	5.2	NM	NM	
Bbr_1847	malE	gcaTGTtAaCGCTCtCAtTg	-247	4.58	++	-	AraQ regulon in Bifidobacteriaceae
Sites found using new PWM							
Bbr_0032	malR5	aAATGatAGCGCTtACATTg	-264	5.14	+	+	AraQ regulon in Bifidobacteriaceae
		aAccGTGACCGCTaACAacg	-158	4.64			Also predicted as MalR5 sites
		gAATGTaAGCGCatACATat	-119	4.65			
Bbr_0033	malE2	atATGTatGCGGTtACATTc	-265	4.65			
		cgTtGTtAGCGgTCACggTt	-226	4.64	NM	NM	
		cAATGTaAGCGGTatCATTt	-120	5.14			
Bbr_0037	icfA	gAtTGTGAGCGtTcTcCATTa	-115	5.6			
Bbr_0038	ahpC	TAATGaaGaaCGCTCACAATc	-173	5.6			
Bbr_0117	agl4	TgATGcataCGCTCgCATTA	-179	4.61	++	++	Also predicted as MalR3 sites
Bbr_0118	malE1	TAATGcGAGCGtatgCATcA	-99	4.61	NM	NM	
Bbr_0176	pbp2	cccTgaGAGCGtTCACgTcA	-55	4.8			
Bbr_0221	bfeU - bfeO	ggATGTtAcCGCTCACATgc	-221	5	NM	NM	
Bbr_0411	araQ	cAATGTGAGCGCTCACgcgA	-112	5.53	+++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_0603	ctr	acATGcGAGCGtTCACgATg	-101	4.87	NM	NM	
Bbr_0704	Bbr_0704	TAATGcaaAaCGtTagCATTA	-69	4.66	NM	NM	
Bbr_0725	eno	cAtTGTGAGCGCTCACATcA	-110	6.32	+++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_0746	glgB - Bbr_745 - Bbr_744	cAATGaaGAGCGCTCACAATc	-108	5.8	+++	++	AraQ regulon in Bifidobacteriaceae
Bbr_0747	carD - ispF	gAtTGTGAGCGGTcTcCATTg	-91	5.8	+++	+++	
Bbr_0757	pyk	cAgTGTGAGCGCTCACAaCg	-130	6.12			AraQ regulon in Bifidobacteriaceae
Bbr_0772	pta - ackA	cAccGTGaaCGaTaACAacg	-76	4.51	NM	NM	AraQ regulon in Bifidobacteriaceae
Bbr_0787	pf1B - pf1A	gAtTggtAGCGCTCACAgAa	-135	5.36	++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_0921	fadD2	cgATGTGaaCGaTtACAATc	-81	4.82	NM	NM	
Bbr_1003	tkt - tal	aAtTGTGaaCGCTaACAgaA	-156	5.45	++	++	AraQ regulon in Bifidobacteriaceae
Bbr_1233	gap	cAATGTGAGCGGTcACAaaA	-156	6.23	+++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_1237	galM	gAtTgcGAGCGgTaACAATA	-46	5.11	NM	NM	AraQ regulon in Bifidobacteriaceae
Bbr_1273	ldh2	ggcTGTGAGCGCTaACAaAct	-160	5.57	+++	+++	AraQ regulon in Bifidobacteriaceae
Bbr_1316	Bbr_1316	ctcTGTtAtCGtTCACAATg	-250	4.88	NM	NM	
Bbr_1419	rbsA1 - rbsC1 - rbsB1 - rbsD1 - rbsK2	gAtTGTtAaCGGTcTcCgacA	-184	4.83	-	++	
Bbr_1650	malQ2	ccaTGTGAGCGGTcTcCAtgt	-138	5.02	NM	NM	AraQ regulon in Bifidobacteriaceae
Bbr_1685	Bbr_1685	ctATGTGaaCGCTcTcCAagc	-232	5.12	NM	NM	
Bbr_1723	birA	acgTGTGAGCGtTCACAaccg	-107	5.2	NM	NM	AraQ regulon in Bifidobacteriaceae
Bbr_1724	Bbr_1724 - Bbr_1725	cggTGTGaaCGCTCACAcgt	-158	5.2	NM	NM	
Bbr_1847	malE	gcaTGTtAaCGCTCtCATTg	-247	5.1	++	-	AraQ regulon in Bifidobacteriaceae
Divergons are marked by thick box borders							
NM = not measured							
New members are in yellow (in comparison with old PWM)							
*Position of a site relative to the first nucleotide of CDS							

Supplemental Table S2. Oligonucleotide primers used in EMSA analysis.

The locus tag number refers to the gene upstream of which a presumed promoter region is present that was amplified by the primer pair. If two locus tags are indicated, it means that the promoter region is located in between two corresponding and divergently oriented genes (i.e., representing an intergenic region). Primers used in the EMSA experiments, Ird represents primers which were labelled with an iridescent probe at the 5' end.

Locus tag	Primer name	Sequence
Bbr_0023	<i>malR2_Ird</i>	gctcgcttttgccatggc
Bbr_0023	<i>malR2</i>	ccgaaaccagccgtgcac
Bbr_0027	<i>malFG 2_Ird</i>	ccatgccggtctccttgc
Bbr_0027	<i>malFG 2</i>	gccagcctactttctctcc
Bbr_0032/Bbr_0033	<i>malR5/malE2_Ird</i>	cgccatgatgtctccttcgc
Bbr_0032/Bbr_0033	<i>malR5/malE2</i>	gctcatgtgatactgcctcc
Bbr_0060	<i>glgP 1_Ird</i>	cattctgacttctctccggg
Bbr_0060	<i>glgP 1</i>	gcgttctatccttgactgagcc
Bbr_0105	<i>cldR_Ird</i>	gctgcgcgctatgttctct
Bbr_0105	<i>cldR</i>	ccacgtcacgaatagtgc
Bbr_0106	<i>cldE_Ird</i>	gcgacgatgacgaatccg
Bbr_0106	<i>cldE</i>	gcacttggcgctcattg
Bbr_0111	<i>agl3_Ird</i>	gttcattcagcccagcgc
Bbr_0111	<i>agl3_Ird</i>	gtcattgaggttgccg
Bbr_0112/Bbr_0113	<i>malR6/Bbr_0113_Ird</i>	cgcttgatgcttgcctttgc
Bbr_0112/Bbr_0113	<i>malR6/Bbr_0113</i>	ccggaacttctcgtatcatcatg
Bbr_0116	<i>malQ 2_Ird</i>	cctgttctgctcttaccgtagctc
Bbr_0116	<i>malQ 2</i>	gagctacggtaagagcagaacagg
Bbr_0117/Bbr_0118	<i>agl4/malFG_Ird</i>	cggttcctacgccaagtaac
Bbr_0117/Bbr_0118	<i>agl4/malFG</i>	aagtgtgctgctgtcatcg
Bbr_0122/Bbr_0123	<i>malR3/apuB_Ird</i>	ggtagatgtctgccttgccc
Bbr_0122/Bbr_0123	<i>malR3/apuB_Ird</i>	gcaagcgcttggcgagcg
Bbr_0411	<i>araQ_Ird</i>	cgcacttctggcatttg
Bbr_0411	<i>araQ</i>	gaacttcaggccgccagc
Bbr_0725	<i>eno_Ird</i>	caaggaagtcgccgacaatc
Bbr_0725	<i>eno</i>	ggttgccacgagaatccag
Bbr_0747	<i>carD_Ird</i>	ggcacattgatgaccagacc
Bbr_0747	<i>carD_Ird</i>	gccgttgattcgtgtgtca
Bbr_0757	<i>pyk_Ird</i>	cgtgagaaggcctgaaatc
Bbr_0757	<i>pyk_Ird</i>	ggttgtcgtaatcctcggtg
Bbr_0787	<i>pflBA_Ird</i>	gccgatagaacagcgtatgg
Bbr_0787	<i>pflBA</i>	cttggcgtcgagctcctcttg
Bbr_0845	<i>glgP 2_Ird</i>	cgcacctcctccacgctg
Bbr_0845	<i>glgP 2</i>	ccgttagattggagattgtccg
Bbr_1002-1003	<i>tkl – tal_Ird</i>	gctcggtctccttgaattcg
Bbr_1002-1003	<i>tkl – tal</i>	ggctcttgccgaacgaatg
Bbr_1233	<i>gap_Ird</i>	gcattgcctcaggaagcc
Bbr_1233	<i>gap</i>	gaccaatgcgaccgaagc
Bbr_1273	<i>ldh_Ird</i>	ggatgtcgattcgacttgg
Bbr_1273	<i>ldh_Ird</i>	agcttgctgttattggtgcc
Bbr_1419	<i>rbsA1_Ird</i>	gctcaatagtccttcgccgcc
Bbr_1419	<i>rbsA1</i>	catacgccctcgtcttcgtc
Bbr_1420	<i>lacI_Ird</i>	gatcatgctcagatgcggcg
Bbr_1420	<i>lacI</i>	cgaatgccataccgtctcc
Bbr_1595	<i>pgma_Ird</i>	ccatacttctcttgcacg
Bbr_1595	<i>pgma</i>	gcgacatcttactccattcc
Bbr_1658	Bbr_1658_Ird	gggtcaagctcatcggtcg
Bbr_1658	Bbr_1658	ctggtcagcatagccgcac
Bbr_1659	<i>LacI_Ird</i>	caacgtgctgcagcctatgg
Bbr_1659	<i>LacI</i>	cttcgccacgtcatacacc

Bbr_1841	Bbr_1841_Ird	cgacagcttccttgccatgc
Bbr_1841	Bbr_1841	gcgtgcatgtccctgatg
Bbr_1845/Bbr_1846	malFG/malR1_Ird	cataacagcccctttgcc
Bbr_1845/Bbr_1846	malFG/malR1	catgactttcctcctcttgag
Bbr_1847	malE_Ird	ggaatgcctgagctgagccg
Bbr_1847	malE	cgaacctttctttcatcgctg
Bbr_1891	gntR_Ird	gatgagtgcgcgtagagaag
Bbr_1891	gntR	cacgctggcggaagattgtc
Bbr_1894	PTS_Ird	gatatgcgcgaggattgg
Bbr_1894	PTS	gatcgacatacagcatgccg
Bbr_1901	nrdH & nrdI & nrdE_Ird	gtctcgaacggcacacca
Bbr_1901	nrdH & nrdI & nrdE	tggacatccggtcaggcc
Generation of DNA fragments for EMSA Fragmentations		
Bbr_0725	eno_1_Ird	caaggaagtcgccgacaatc
Bbr_0725	eno_2	gggtgtgccgcgtgattgc
Bbr_0725	eno_3	ccaaaatttgatgtgagcgctc
Bbr_0725	eno_4	ggttgccacgagaatccag
Bbr_0757	pyk_1_Ird	cgctgagaaggcctgaaatc
Bbr_0757	pyk_2	gcgccgattgggtttgag
Bbr_0757	pyk_3_Ird	ccgggtgtgttgtagcgc
Bbr_0757	pyk_4_Ird	ggttgtcgaatcctcggtg
Bbr_0032/Bbr_0033	malR5/malE2_1_Ird	cgccatgatgtctctttcgc
Bbr_0032/Bbr_0033	malR5/malE2_2	caacgcgcacatcgtggtag
Bbr_0032/Bbr_0033	malR5/malE2_3_Ird	gtaccacgatgtgcgcgttg
Bbr_0032/Bbr_0033	malR5/malE2_4	cacaccgtcaaccgccgc
Bbr_0032/Bbr_0033	malR5/malE2_5	gcggcggttgacgggtgtg
Bbr_0032/Bbr_0033	malR5/malE2_6	gctcatgtgatactgcctcc
Bbr_0032/Bbr_0033	malR5/malE2_7_Ird	gcagccggcatccgatcc
Bbr_0112/Bbr_0113	malR6/agl3_1_Ird	cgcttgaaatgcttctttgc
Bbr_0112/Bbr_0113	malR6/agl3_2	gacaatcgaaacgcacacacc
Bbr_0112/Bbr_0113	malR6/agl3_3	ccaccgggcatgatacc
Bbr_0112/Bbr_0113	malR6/agl3_4	ccatgtcggcgaatttcctc
Bbr_0112/Bbr_0113	malR6/agl3_5	ccatgctattatgaaacgatgtcag
Bbr_0112/Bbr_0113	malR6/agl3_6_Ird	ccggaacttctcgctatcatcatg
Bbr_0122/Bbr_0123	malR3/apuB_1_Ird	ggtagatgtctgccttgccc
Bbr_0122/Bbr_0123	malR3/apuB_2	gattacacatcgtggatggcgc
Bbr_0122/Bbr_0123	malR3/apuB_3_Ird	gcgccatccacgatgtgaatc
Bbr_0122/Bbr_0123	malR3/apuB_4	gggaagtgttgcttggtgtgg
Bbr_0122/Bbr_0123	malR3/apuB_5	ccacaccaagcaacacttccc
Bbr_0122/Bbr_0123	malR3/apuB_6	cggcatgcagcacagttgac
Bbr_0122/Bbr_0123	malR3/apuB_7_Ird	gtcaactgtgctgcatgccg
Bbr_0122/Bbr_0123	malR3/apuB_8	ctgaccgtgcgtagggg
Bbr_0122/Bbr_0123	malR3/apuB_9	cccctatcgacggtcag
Bbr_0122/Bbr_0123	malR3/apuB_10_Ird	gcaaggcggttggcgagcg

Supplemental Table S3. Location of the AraQ/MalR1 TFBS in relation to the -10/-35 sites. The distance in base pairs [bp] is measured from the 3' -end of the TFBS to the 5' -end of the predicted -35 sequence.

Locus Tag Bbr_	Gene Name	Activator/ Repressor/ Unknown	Distance between TFBS and predicted -35 [bp]
0725	<i>eno</i>	Activator	16
0747	<i>carD</i>	Possible Repressor	-1 (overlaps with -35)
0757	<i>pyk</i>	Activator	15
0787	<i>pfl</i>	Activator	33
1002	<i>tkl</i>	Activator	6
1233	<i>gap</i>	Activator	35
1273	<i>ldh2</i>	Activator	65
1847	<i>malE</i>	Activator	92
1723	-	Activator	41
0037	<i>icfA</i>	Activator	34
0038	<i>ahpC</i>	Unknown	84
0117	<i>agl4</i>	Repressor	-9 (overlaps with -35)
0060	<i>glgP</i>	Repressor	-13 (overlaps with -10)

Supplemental Table S4. Effector molecules tested

Effector Molecule
Maltose
Maltotriose
Maltulose
Isomaltose
Lactose
Glucose
Galactose
Sucrose
Trehalose
Glucose 6-phosphate
Glucose 1-phosphate
Pyruvic acid
Sodium Acetate
Fructose 6-phosphate
Cellobiose
Palatinose
Turanose
DL-Glyceraldehyde 3-phosphate
Acetyl coenzyme A sodium salt
Phosphoenolpyruvate
Acetyl Phosphate
D-Sedoheptulose-7-phosphate
D-Ribose 5-phosphate disodium salt
D-(-)-3-Phosphoglyceric acid disodium salt
Butyrate
Lactate
Propionate
Acetate
Acetyl Aldehyde
1,2 Propanediol
D-erythrose-4-Phosphate
Oxaloacetic acid
Cyclic-AMP
Succinic acid
D-Ribulose 5-phosphate disodium salt

Supplemental Table S5.

Transcriptomic analysis of *B. breve* UCC2003-*araQ* (Up-regulation)

Global transcriptomic analysis of *B. breve* UCC2003-*araQ* mutant as compared with *B. breve* UCC2003 when grown on mMRS supplemented with ribose (up-regulated gene above a 2-fold threshold are indicated). The level of expression is shown as a fold-value of increase in expression, with a cut-off of a minimum >2-fold increase in expression.

Locus tag	Up	Gene name and/or predicted Function	P value
Bbr_0026	2.2	Permease protein of ABC transporter system for sugars	4.50E-01
Bbr_0027	2.2	Permease protein of ABC transporter system for sugars	4.53E-01
Bbr_0030	2.7	Conserved hypothetical protein	3.67E-01
Bbr_0060	2.6	<i>glgP1</i> , Glycogen phosphorylase	3.78E-01
Bbr_0110	6.5	<i>ilvC2</i> , Ketol-acid reductoisomerase/2-dehydropantoate 2-reductase	1.54E-01
Bbr_0116	2.5	<i>malQ</i> , 1 4-alpha-glucanotransferase	3.98E-01
Bbr_0117	2.8	<i>agl4</i> , Alpha-glucosidase	3.59E-01
Bbr_0118	4.4	<i>malE1</i> , Maltose/maltodextrin-binding protein	2.25E-01
Bbr_0119	4.4	<i>malF1</i> , Maltodextrin transport system permease protein	2.28E-01
Bbr_0120	4.5	<i>malG1</i> , Maltose transport system permease protein	2.21E-01
Bbr_0121	3.9	Conserved hypothetical membrane spanning protein	2.59E-01
Bbr_0122	3.7	<i>malR3</i> , Transcriptional regulator, LacI family	2.73E-01
Bbr_0123	4.3	<i>apuB</i> , Amylopullulanase	2.34E-01
Bbr_0164	4.3	<i>oppA1</i> Oligopeptide-binding protein	2.35E-01
Bbr_0165	4.6	<i>oppB1</i> , Oligopeptide transport system permease protein	2.18E-01
Bbr_0171	2.2	Sialidase A	4.60E-01
Bbr_0284	2.5	Sugar/Sodium symporter	4.04E-01
Bbr_0285	2.3	<i>lacZ2</i> , Beta-galactosidase	4.34E-01
Bbr_0538	2.2	<i>cysK</i> , cysteine synthase	4.63E-01
Bbr_1416	2.0	<i>rbsD</i> , D-Ribose pyranase	4.95E-01
Bbr_1429	2.1	<i>cbiO2</i> , Cobalt transport ATP-binding protein	4.70E-01
Bbr_1430	2.1	<i>cbiQ</i> , Cobalt transport protein	4.81E-01
Bbr_1530	2.8	Conserved hypothetical protein with CHAP and transglycosylase SLT	3.62E-01
Bbr_1644	2.3	Narrowly conserved hypothetical membrane spanning protein	4.35E-01
Bbr_1742	2.3	L-fucose permease	4.37E-01
Bbr_1743	2.3	Short chain dehydrogenase	4.38E-01
Bbr_1842	2.2	<i>aap6</i> , Amino acid permease	4.64E-01
Bbr_1845	2.4	Permease protein of ABC transporter system for sugars	4.24E-01
Bbr_1889	5.1	Cell surface protein with gram positive anchor domain	1.95E-01
Bbr_1890	6.2	ATP-binding protein of ABC transporter system for sugars	1.62E-01
Bbr_1891	7.6	Transcriptional regulator, GntR family	1.32E-01
Bbr_1892	8.6	PTS system, IIC component	1.16E-01

Supplemental Table S6.**Transcriptomic analysis of *B. breve* UCC2003-*araQ* (Down-regulation)**

Global transcriptomic analysis of *B. breve* UCC2003-*araQ* mutant as compared with *B. breve* UCC2003 when grown on mMRS supplemented with ribose (down regulated gene above a 2-fold threshold are indicated). The level of expression is shown as a fold-value of increase in expression, with a cut-off of a minimum >2-fold increase in expression.

Locus tag	Down	Gene name and/or predicted Function	P value
Bbr_0104	4.0	<i>ilvC1</i> , Ketol-acid reductoisomerase/2-dehydropantoate 2-reductase	0.00E+00
Bbr_0113	3.6	Cell surface protein precursor with Cna protein B-type domain and	1.43E-08
Bbr_0114	2.3	Cell surface protein with Gram positive anchor and Cna protein B-type domain	0.00E+00
Bbr_0115	2.4	Sortase	1.48E-05
Bbr_0267	2.1	<i>glpF</i> , Glycerol uptake facilitator protein	7.11E-15
Bbr_0366	7.3	Narrowly conserved hypothetical secreted protein with Gram	0.00E+00
Bbr_0368	2.8	Conserved hypothetical protein	1.10E-08
Bbr_0441	14.1	Capsular polysaccharide biosynthesis protein	1.17E-11
Bbr_0442	11.7	Capsular polysaccharide biosynthesis protein	3.77E-15
Bbr_0443	6.3	Glycosyltransferase	1.66E-12
Bbr_0444	41.2	Membrane spanning polysaccharide biosynthesis protein	0.00E+00
Bbr_0445	8.7	Glycosyltransferase	0.00E+00
Bbr_0446	14.9	Acetyltransferase (cell wall biosynthesis)	2.33E-12
Bbr_0447	6.5	Conserved hypothetical protein	1.22E-15
Bbr_0448	4.3	Glycosyltransferase	4.05E-07
Bbr_0449	5.2	Hypothetical membrane spanning protein	5.71E-14
Bbr_0450	2.7	Membrane spanning protein involved in polysaccharide biosynthesis	1.44E-08
Bbr_0532	6.1	Transcriptional regulator, homologs of Bvg accessory factor	4.44E-16
Bbr_0533	3.3	Solute-binding protein of ABC transporter system for peptides	0.00E+00
Bbr_0534	2.7	Permease protein of ABC transporter system for peptides	0.00E+00
Bbr_0535	2.6	Permease protein of ABC transporter system for peptides	0.00E+00
Bbr_0536	3.0	ATP-binding protein of ABC transporter system for peptides	3.38E-12
Bbr_0602	4.0	Low specificity-threonine aldolase	1.19E-08
Bbr_0610	2.1	Conserved hypothetical membrane spanning protein	7.10E-08
Bbr_0611	2.4	Narrowly conserved hypothetical protein	3.37E-07
Bbr_0612	2.1	<i>crcB</i> , family protein	1.98E-07
Bbr_0674	2.4	Peptidase family M20A protein	7.44E-15
Bbr_0675	2.6	Permease protein of ABC transporter system	6.63E-11
Bbr_0889	2.3	Glutamine amidotransferase	1.48E-13
Bbr_0924	2.6	pntB NAD(P) transhydrogenase subunit beta	0.00E+00
Bbr_1078	2.1	Lantibiotic transport ATP-binding protein	3.23E-04
Bbr_1327	6.7	dTDP-rhamnosyl transferase	0.00E+00
Bbr_1328	4.2	Conserved hypothetical membrane spanning protein	0.00E+00
Bbr_1364	2.3	<i>groEL</i> , 60 kDa chaperonin	0.00E+00
Bbr_1474	3.0	Sua5/YciO/YrdC/YwlC family protein	0.00E+00
Bbr_1475	2.0	<i>livF</i> , Branched-chain amino acid transport ATP-binding protein	8.88E-16
Bbr_1476	2.1	<i>livG</i> , Branched-chain amino acid transport ATP-binding protein	2.47E-12

Bbr_1477	2.0	<i>livM</i> , Branched-chain amino acid transport system permease protein	3.12E-11
Bbr_1478	2.6	<i>livH</i> , Branched-chain amino acid transport system permease protein	5.95E-14
Bbr_1642	2.2	<i>rpsJ</i> , 30S ribosomal protein	1.58E-12
Bbr_1718	2.1	Hypothetical protein	5.55E-16
Bbr_1719	2.3	<i>fas</i> , Type I multifunctional fatty acid synthase	0.00E+00
Bbr_1720	2.1	<i>accD</i> , Acetyl-/propionyl-CoA carboxylase beta chain	2.22E-16
Bbr_1884	2.3	<i>galT2</i> , Galactose-1-phosphate uridylyltransferase	4.19E-06
Bbr_1886	2.1	Narrowly conserved hypothetical secreted protein	9.42E-12

Supplemental Table S7.**Transcriptomic analysis of *B. breve* UCC2003-*malR1* (Up-regulation)**

Global transcriptomic analysis of *B. breve* UCC2003-*malR1* mutant as compared with *B. breve* UCC2003 when grown on mMRS supplemented with ribose (up-regulated gene above a 1.2-fold threshold are indicated). The level of expression is shown as a fold-value of increase in expression, with a cut-off of a minimum >1.2-fold increase in expression.

Locus tag	Up	Gene name and/or predicted Function	P value
Bbr_0118	2	<i>malE1</i> , Maltose/maltodextrin-binding protein	3.61E-13
Bbr_0119	1.3	<i>malF1</i> , Maltodextrin transport system permease protein	2.46E-09
Bbr_0123	1.8	<i>apuB</i> , Amylopullulanase	6.34E-12
Bbr_0299	1.2	Holin	2.45E-07
Bbr_0391	1.2	<i>ilvB</i> , Acetolactate synthase large subunit	3.24E-07
Bbr_0558	1.3	Transcriptional regulator, LacI family	4.66E-12
Bbr_0607	1.4	<i>Tuf</i> , Protein Translation Elongation Factor Tu (EF-TU)	3.61E-03
Bbr_0725	1.3	<i>eno</i> , Enolase	2.93E-04
Bbr_0776	1.5	Xylulose-5-phosphate/Fructose-6-phosphate phosphoketolase	4.61E-06
Bbr_0787	1.2	<i>pfl</i> , Formate acetyltransferase	1.36E-01
Bbr_0847	1.3	<i>nagB2</i> , Glucosamine-6-phosphate isomerase	3.99E-10
Bbr_0848	1.3	Sugar kinase, ROK family	4.91E-06
Bbr_0921	1.3	<i>fadD2</i> , Long-chain-fatty-acid--CoA ligase	4.65E-09
Bbr_0969	1.3	<i>metE</i> , 5-methyltetrahydropteroyltri-glutamate—homocysteine methyltransferase	6.99E-11
Bbr_0970	1.3	<i>metF</i> , Methylenetetrahydrofolate reductase	1.28E-12
Bbr_0973	1.3	<i>pyrB</i> , Aspartate carbamoyltransferase	5.89E-11
Bbr_1003	1.3	<i>tkt</i> , Transketolase	5.39E-04
Bbr_1079	1.3	Two-component response regulator	7.20E-11
Bbr_1233	1.3	Glyceraldehyde 3-phosphate dehydrogenase	4.01E-03
Bbr_1273	1.2	<i>ldh2</i> , L-lactate dehydrogenase	3.43E-04
Bbr_1367	1.3	<i>ung</i> , Uracil-DNA glycosylase	3.31E-11
Bbr_1505	1.2	<i>fucO</i> , Lactaldehyde reductase	2.05E-05
Bbr_1537	1.3	Hypothetical protein	2.53E-11
Bbr_1635	1.3	<i>rpsC</i> , 30S ribosomal protein	9.91E-03
Bbr_1649	1.3	<i>rplM</i> , 50S ribosomal protein L13	2.09E-02
Bbr_1719	1.2	<i>fas</i> , Type I multifunctional fatty acid synthase	6.48E-08
Bbr_1909	1.3	Conserved hypothetical protein	3.96E-08

Supplemental Table S8.

Transcriptomic analysis of *B. breve* UCC2003-*malR1* (Down-regulation)

Global transcriptomic analysis of *B. breve* UCC2003-*malR1* mutant as compared with *B. breve* UCC2003 when grown on mMRS supplemented with ribose (down-regulated gene above a 1.2-fold threshold are indicated). The level of expression is shown as a fold-value of increase in expression, with a cut-off of a minimum >1.2-fold increase in expression.

Locus tag	Down	Gene name and/or predicted Function	P value
Bbr_0112	1.8	<i>malR6</i> , Transcriptional regulator, LacI family	7.65E-07
Bbr_0030	1.3	Conserved hypothetical proetin	1.63E-01
Bbr_0044	1.3	<i>pelF</i> , Glycosyl transferase (Polysaccharide biosynthesis protein)	1.65E-08
Bbr_0045	1.2	Conserved hypothetical membrane spanning protein	4.09E-09
Bbr_0107	1.2	<i>cebF</i> , Cellobiose/cellotriose transport system permease protein	8.37E-11
Bbr_0468	1.2	Hypothetical protein	3.17E-10
Bbr_0535	1.3	Permease protein of ABC transporter system for peptides	5.61E-11
Bbr_1299	1.2	<i>ispA</i> , Lipoprotein signal peptidase	3.42E-07
Bbr_1482	1.3	<i>rpmE2</i> , LSU ribosomal protein L31P	2.97E-06
Bbr_1597	1.3	<i>rrf2</i> , family protein	3.53E-09
Bbr_1598	1.4	Pyridine nucleotide-disulphide oxidoreductase family protein	2.38E-14
Bbr_1658	1.2	Sugar-binding protein of ABC transporter system	2.32E-08
Bbr_1667	1.5	<i>rpmG</i> , LSU ribosomal protein L33P	3.67E-06
Bbr_1668	1.4	<i>Hsp10</i> , 10 kDa chaperonin GROES	1.24E-08
Bbr_1669	1.3	Narrowly conserved hypothetical membrane spanning protein	2.92E-10
Bbr_1731	1.3	<i>aspC</i> , Aspartate aminotransferase	3.75E-10
Bbr_1774	1.7	Transposase	3.19E-09
Bbr_1828	1.2	Transcriptional regulator, MarR family	4.07E-10
Bbr_1843	1.2	Narrowly conserved hypothetical membrane spanning protein	1.76E-07
Bbr_1844	1.3	Permease protein of ABC transporter system for sugars	2.67E-07
Bbr_1845	1.4	Permease protein of ABC transporter system for sugars	2.95E-08
Bbr_1854	1.3	<i>tdcB</i> , Threonine dehydratase	2.88E-10
Bbr_1873	1.3	Phospholipase/carboxylesterase	1.74E-11
Bbr_1890	1.2	ATP-binding protein of ABC transporter system for sugars	5.35E-04
Bbr_1905	1.3	Narrowly conserved hypothetical protein	7.05E-08
Bbr_1914	1.3	<i>pcnA</i> , tRNA nucleotidyl transferase	4.98E-09
Bbr_1918	1.2	<i>trxB2</i> , Thioredoxin reductase	3.41E-08
Bbr_1920	1.3	<i>parA</i> , Chromosome partitioning protein	7.06E-11
Bbr_1926	1.3	<i>rpmH</i> , LSU Ribosomal protein	6.81E-03

Supplemental Table S9.

Distribution of *araQ* and *malR1* homologues among Bifidobacteriaceae members

Strain	<i>araQ</i>	<i>malR1</i>
<i>Aeriscardovia aeriphila</i> LMG 21773	+	0
<i>Alloiscardovia criceti</i> DSM 17774 (<i>Metascardovia criceti</i> DSM 17774)	+	+
<i>Alloiscardovia macacae</i> DSM 24762	+	+
<i>Alloiscardovia omnicoles</i> F0580	+	+
<i>Bifidobacterium actinocoloniiforme</i> DSM 22766	+	0
<i>Bifidobacterium adolescentis</i> ATCC 15703	+	+
<i>Bifidobacterium aesculapii</i> DSM 26737 (DSM 26737T)	+	+
<i>Bifidobacterium angulatum</i> DSM 20098	+	+
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> AD011	+	+
<i>Bifidobacterium animalis</i> subsp. <i>animalis</i> LMG 10508	+	+
<i>Bifidobacterium aquikefiri</i> LMG 28769	+	+(2x)
<i>Bifidobacterium asteroides</i> PRL2011	+	0
<i>Bifidobacterium biavatii</i> DSM 23969	+	0
<i>Bifidobacterium bifidum</i> NCIMB 41171	+	0
<i>Bifidobacterium bohemicum</i> DSM 22767	+	0
<i>Bifidobacterium bombi</i> DSM 19703	+	0
<i>Bifidobacterium boum</i> LMG 10736	+	+
<i>Bifidobacterium breve</i> UCC2003	+	+
<i>Bifidobacterium callitrichos</i> DSM 23973	+	+
<i>Bifidobacterium catenulatum</i> DSM 16992 = JCM 1194	+	+
<i>Bifidobacterium choerinum</i> LMG 10510	+	+
<i>Bifidobacterium commune</i> R-52791	+	0
<i>Bifidobacterium coryneforme</i> LMG 18911	+	+
<i>Bifidobacterium crudilactis</i> LMG 23609	+	+
<i>Bifidobacterium cuniculi</i> LMG 10738	+	+
<i>Bifidobacterium dentium</i> Bd1	+	+
<i>Bifidobacterium eulemuris</i> DSM 100216	+	+
<i>Bifidobacterium gallicum</i> DSM 20093	+	+
<i>Bifidobacterium gallinarum</i> LMG 11586	+	0
<i>Bifidobacterium hapali</i> DSM 100202	+	0
<i>Bifidobacterium indicum</i> LMG 11587 = DSM 20214	+	+
<i>Bifidobacterium kashiwanohense</i> JCM 15439 = DSM 21854	+	+
<i>Bifidobacterium lemum</i> DSM 28807	+	+
<i>Bifidobacterium longum</i> subsp. <i>infantis</i> ATCC 15697 = JCM 1222	+	+
<i>Bifidobacterium longum</i> subsp. <i>longum</i> NCC2705	+	+
<i>Bifidobacterium longum</i> subsp. <i>suis</i> LMG 21814	+	+
<i>Bifidobacterium magnum</i> LMG 11591	+	+
<i>Bifidobacterium merycicum</i> LMG 11341	+	+
<i>Bifidobacterium minimum</i> LMG 11592	+	+
<i>Bifidobacterium mongoliense</i> DSM 21395	+	+
<i>Bifidobacterium moukalabense</i> DSM 27321	+	+
<i>Bifidobacterium myosotis</i> DSM 100196	+	+
<i>Bifidobacterium pseudocatenulatum</i> DSM 20438 = JCM 1200 = LMG 10505	+	+
<i>Bifidobacterium pseudolongum</i> PV8-2	+	+
<i>Bifidobacterium pseudolongum</i> subsp. <i>globosum</i> LMG 11569	+	+
<i>Bifidobacterium pseudolongum</i> subsp. <i>pseudolongum</i> LMG 11571	+	+
<i>Bifidobacterium psychraerophilum</i> LMG 21775	+	+
<i>Bifidobacterium pullorum</i> DSM 20433	+	0
<i>Bifidobacterium reuteri</i> DSM 23975	+	+
<i>Bifidobacterium ruminantium</i> LMG 21811	+	+
<i>Bifidobacterium saeculare</i> DSM 6531 = LMG 14934	+	0
<i>Bifidobacterium saguini</i> DSM 23967	+	+
<i>Bifidobacterium scardovii</i> JCM 12489 = DSM 13734	+	+
<i>Bifidobacterium stellenboschense</i> DSM 23968	+	+(2x)
<i>Bifidobacterium stercoris</i> JCM 15918	+	+(2x)
<i>Bifidobacterium subtile</i> LMG 11597	+	0
<i>Bifidobacterium thermacidophilum</i> subsp. <i>porcinum</i> LMG 21689	+	+
<i>Bifidobacterium thermacidophilum</i> subsp. <i>thermacidophilum</i> DSM 15837	+(2x)	+
<i>Bifidobacterium thermophilum</i> RBL67	+	+
<i>Bifidobacterium thermophilum</i> DSM 20212	+	+
<i>Bifidobacterium tissieri</i> DSM 100201	+	0
<i>Bifidobacterium tsurumiense</i> JCM 13495	+	+
<i>Bombiscardovia coagulans</i> DSM 22924	+	0
<i>Gardnerella vaginalis</i> 409-05	+	+
<i>Gardnerella vaginalis</i> ATCC 14019	0	+
<i>Parascardovia denticolens</i> DSM 10105 = JCM 12538	+	0
<i>Pseudoscardovia radai</i> DSM 24742	0	0
<i>Pseudoscardovia suis</i> DSM 24744	0	0
<i>Scardovia inopinata</i> JCM 12537 (<i>Scardovia inopinata</i> F0304)	0	0
<i>Scardovia wiggisiae</i> F0424	+	0
Total number of genomes with a gene	66	50
2x means 2 copies of a gene are present in a genome		

Supplemental Table S10. Oligonucleotide primers used in this study.

Purpose	Primer	Sequence
Cloning of internal fragment of <i>malR1</i> in pORI19	<i>malR1_IM_</i>	ttgctaaagcttgaaatcaggccgatcactgcg
Cloning of internal fragment of <i>araQ</i> in pORI19	<i>malR1_IM_</i>	ttgctatctagacaatatcgcggtggccaagg
	<i>araQ_IM_F</i>	atagctaagcttgattcgccggatgtgtctg
	<i>araQ_IM_R</i>	atagcttctagagtacagggcggacttatggc
Confirmation of site specific homologous recombination	<i>malR1_Con</i>	gccatcgccgacgaactc
	<i>araQ_Con</i>	gtgtgcaggccgccattg
	<i>tetW_F</i>	tcagctgtcgactgctcatgtacggtaag
	<i>tetW_R</i>	gcgacggtcgaccattaccttctgaaacat
Cloning of <i>malR1</i> in pQE60	<i>malR1_F</i>	ttgctaccatggatgaccacaagtatccaagatgtcgcc
	<i>malR1_R</i>	ttgctaggatccgcggatcttggcggtagagg
Cloning of <i>araQ</i> in pNZ44	<i>araQ_F</i>	atagcttctagaatggagggttcgggcagtatggtg
	<i>araQ_R_hist</i>	atagctaagcttttacatcaccatcaccatcaccatcaccatcacgaacggcactcagcacagc
Purpose	Primer	Sequence
Cloning of internal fragment of <i>malR1</i> in pORI19	<i>malR1_IM_</i>	ttgctaaagcttgaaatcaggccgatcactgcg
Cloning of internal fragment of <i>araQ</i> in pORI19	<i>malR1_IM_</i>	ttgctatctagacaatatcgcggtggccaagg
	<i>araQ_IM_F</i>	atagctaagcttgattcgccggatgtgtctg
	<i>araQ_IM_R</i>	atagcttctagagtacagggcggacttatggc
Confirmation of site specific homologous recombination	<i>malR1_Con</i>	gccatcgccgacgaactc
	<i>araQ_Con</i>	gtgtgcaggccgccattg
	<i>tetW_F</i>	tcagctgtcgactgctcatgtacggtaag
	<i>tetW_R</i>	gcgacggtcgaccattaccttctgaaacat
Cloning of <i>malR1</i> in pQE60	<i>malR1_F</i>	ttgctaccatggatgaccacaagtatccaagatgtcgcc
	<i>malR1_R</i>	ttgctaggatccgcggatcttggcggtagagg
Cloning of <i>araQ</i> in pNZ44	<i>araQ_F</i>	atagcttctagaatggagggttcgggcagtatggtg
	<i>araQ_R_hist</i>	atagctaagcttttacatcaccatcaccatcaccatcaccatcacgaacggcactcagcacagc

Supplemental Figures

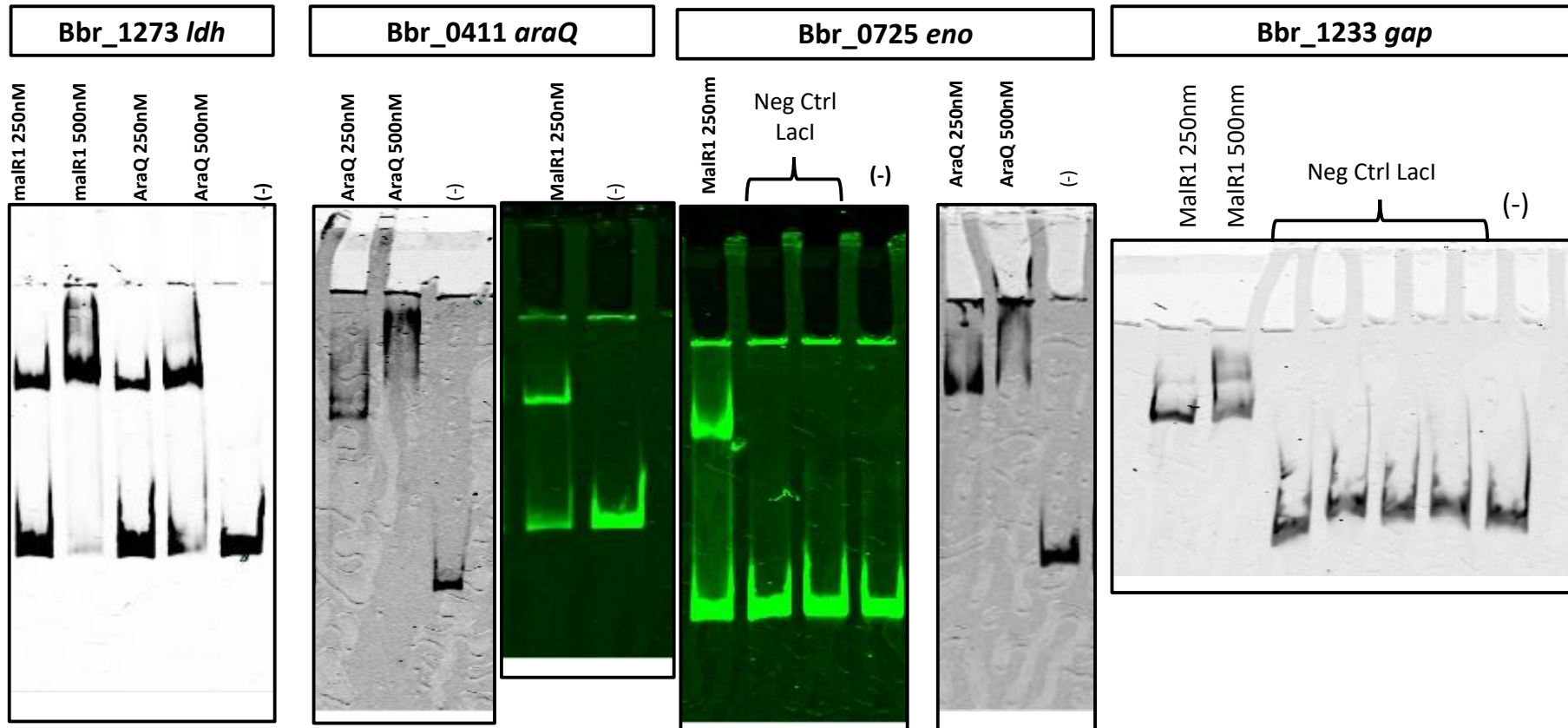
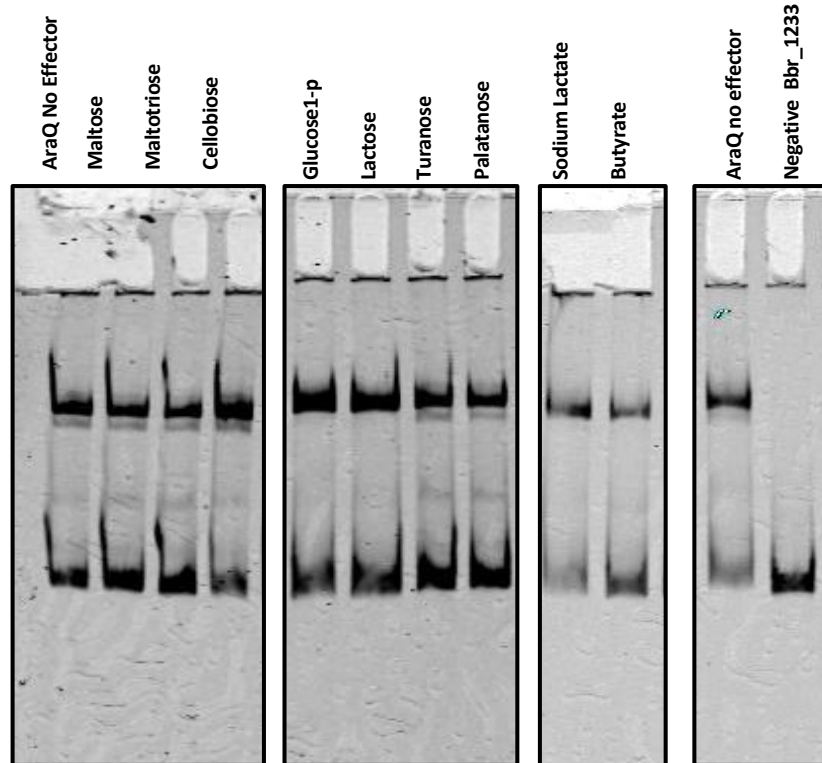


Figure S1. Example EMSA

All EMSA analysis carried out in the above figure were carried out with 0 nM (-), 250 nM or 500 nM of AraQ or MalR1 protein. These proteins were incubated with 0.5 nM Ird labelled DNA fragments encompassing the promoter region of the specified gene whose locus tag and gene name are indicated above each image in bold.

AraQ



MalR1

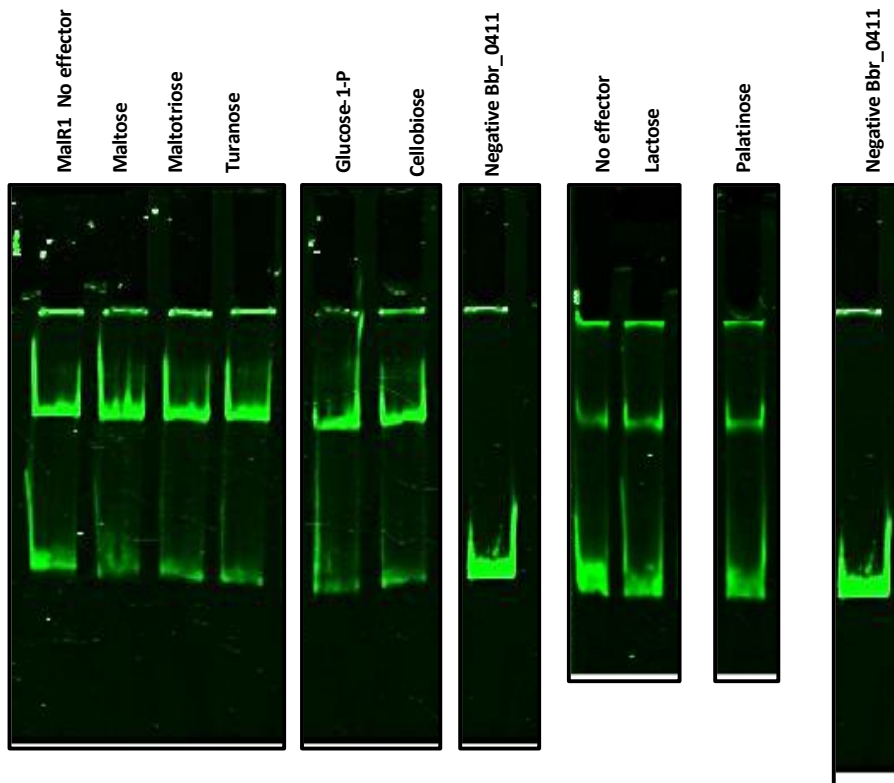
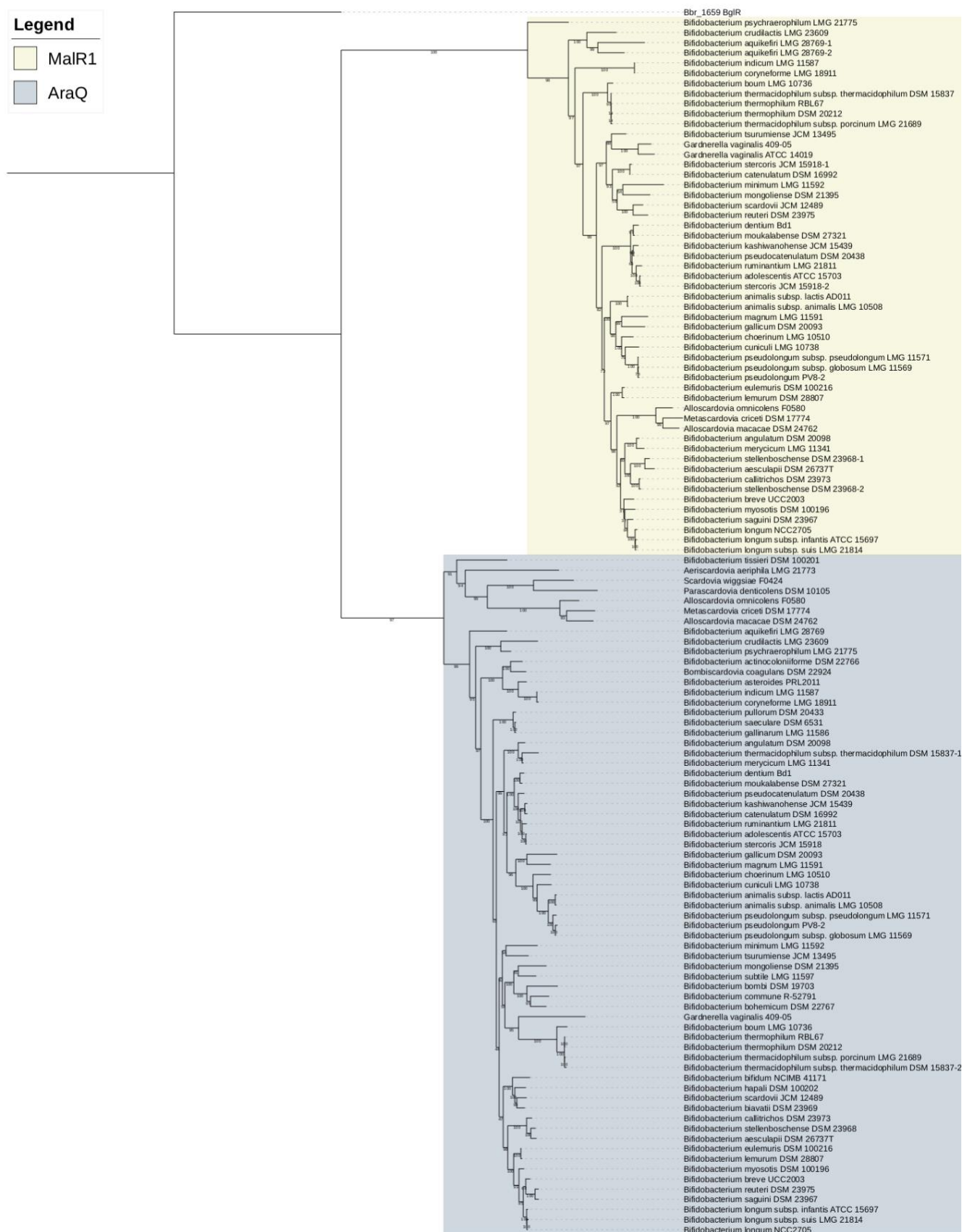


Figure S2. Example EMSA of AraQ and MalR1 Effector Assays

All EMSA analysis were carried out with 150 nM protein (AraQ or MalR1) or 0 nM protein (Neg.), 10mM Effector Listed above and with 0.5 nM Ird labelled DNA fragments encompassing the promoter region of the specified gene.

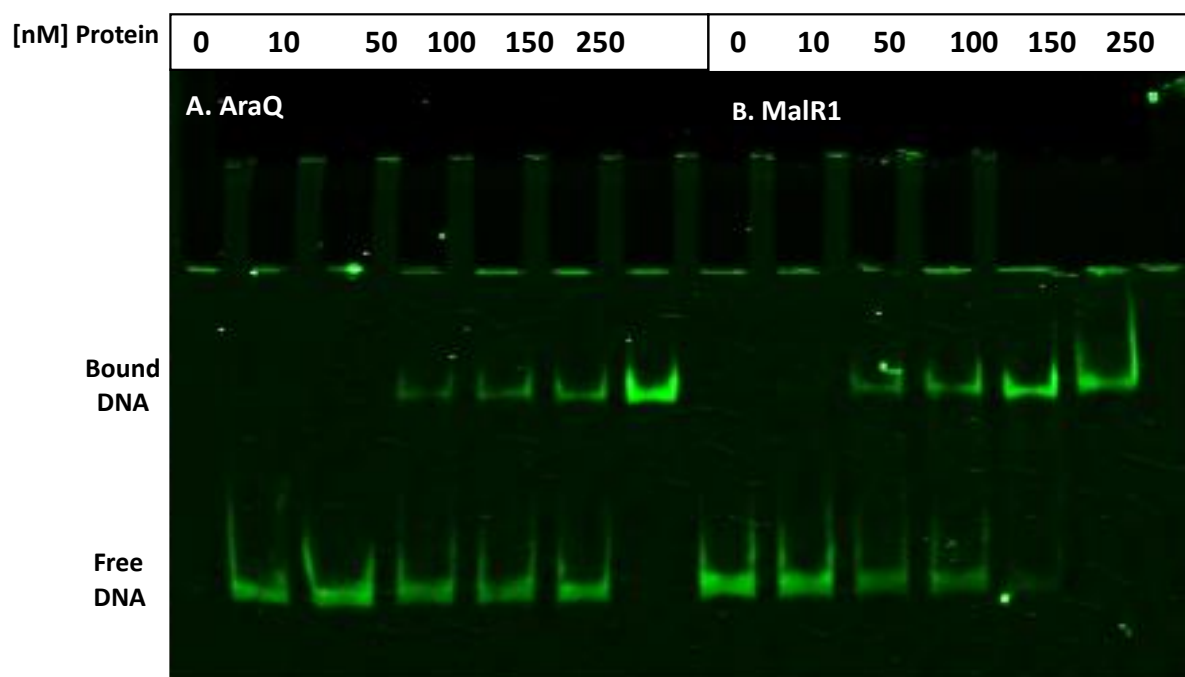
Tree scale: 1

Legend



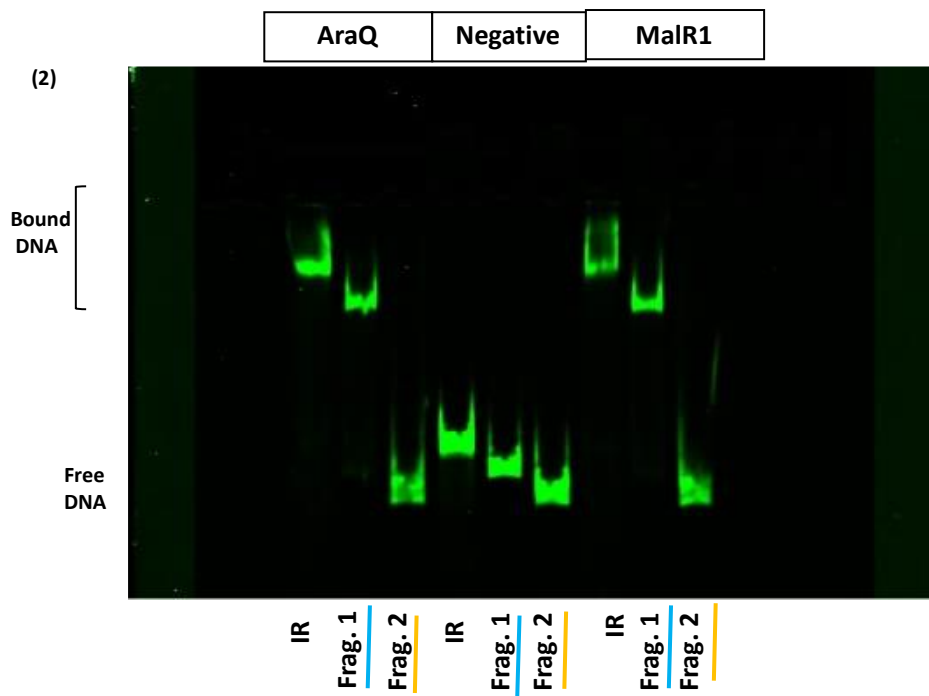
Supplemental Figure S3. Phylogenetic analysis of AraQ and MalR1

The analysis was carried out utilising an ultrafast bootstrap with 1000 replicates. BglR (Bbr_1659) a distant LacI family regulator from *B. breve* UCC2003, was utilised as an out group.



Supplemental Figure S4. Uncropped Image of gel in Figure 1.

EMSA carried out utilising Increasing concentrations (0, 10, 50, 100, 150, 250nM purified protein) of purified AraQ (A) and MalR1 (B) incubated with 0.5nM Ird labelled DNA fragment encompassing the Bbr_1233 promoter region.



Supplemental Figure S5. Uncropped gel image of gel in Figure 3 Panel (2)

EMSA to investigate AraQ and MalR1 abilities to bind to Bbr_0757 promoter region fragments (IR, Frag 1 and Frag 2). All reactions contain 0.5 nM Ird labelled DNA and 150 nM of either AraQ or MalR1 protein, while negative reactions contain 0 nM protein.