Table S1 Oligonucleotide primers used in this study (primer pairs indicated in materials and methods)

Primer	Sequence	Size bp
melR1fHd3	TGCGGAAAGCTTATTCTTTACACGGTTACC	380
melR1rxba1	CTATGCTCTAGACGATGACCTGTTTGGCCGA	
melR2fHd3	TGCGGAAAGCTTACTTGCTATCATACCGAC	402
melR2rxba1	CTATGCTCTAGACCTGTACTGCGATGATGT	
rafRfHd3	GCTAAAGCTTGCAGACCGCATT	530
rafRrxba1	GTCATCTAGAGCACATGCCCCACTAGGC	
melR1-confirm	GCTCGCGAACGGCTGGC	2600
Tetwsal1F	TCAGCTGTCGACATGCTCATGTACGGTAAG	
melR2-confirm	TCACTGGCTGGCTGGTTC	2600
Tetwsal1F	TCAGCTGTCGACATGCTCATGTACGGTAAG	
rafR-confirm	GCATAGATCTTCTGACGCCAATGCGATTCC	2600
Tetwsal1F	TCAGCTGTCGACATGCTCATGTACGGTAAG	
Tetwsal1R	GCGACGGTCGACCATTACCTTCTGAAACATA	
melR1EcorVF	GACAAGGATATCATGCATCACCATCACCATCACCATCACCATCACAAACGCGCGACCATCAAC	1001
melR1Xba1	GACAAGTCTAGACTATTCCCGCTCTGGCCC	
melR2Nco1F	TGCACGCCATGGGCCATCACCATCACCATCACCATCACCATCACAGCGAACCAACAATCTATG	1002
melR2Xba1R	CTATGCTCTAGATTAATCTCGAGGTGCTGG	
rokFfbglII	GCATAGATCTTCTGACGCCAATGCGATTCC	
rokRrpst1	CGTACTGCAGCCTACCCAATATGCTTCAC	1206
rafAPEFDNA	CTGAAGTGCATGGCGGCG	
rafAPERDNA	GCATCATACGCAGCGAGCAAG	378
rafBPEFDNA	CATTGGCGTCAGACATACTTAAG	
rafBPERDNA	GAGAACCCGCCGACCACG	735
melAPEFDNA	AGCCTTCGAAACGTCATCC	
melAPERDNA	GGTGATGTTCGGGTACT	514
1862PEFDNA	GATTGTTGGTTCGCTCATGGG	
1862PERDNA	CTTTGATGGTGATGTTTG	471

Table S2 IRD800 primers used to generate PCR products and primer extension products in this study (primer pairs indicated in materials and methods)

Name	Primer	Product size (bp)
melAIRD800f	AGCCTTCGAAACGTCATCC	
melAIRD800r	CAGACATCAGACCGGCAATAAC	322
melAIRD800SET1F	TCGCGAGAAGGAAGTGC	
melAIRD800SET1R	GTTGGCTTTGTTGCTGGA	312
melAIRD800SET2F	TCGGCCATTTGTTTGCG	
melAIRD800SET2R	GCCAGCGCCGTCCCACA	302
melAIRD800SET3F	TAAGCCGAGATAGCCAAG	
melAIRD800SET3R	GGTGATGTTCGGGTACT	302
1862IRD800irdf	GATTGTTGGTTCGCTCATGGG	
1862IRD800irdr	GAGCCAGGACCACATGCAGT	310
1862IRD800SET1F	GAAGTTAAAGTCGCCATTG	
1862IRD800SET1R	CTAAATCAGTGCTGACATC	300
1862IRD800SET2F	CGTAATCGATATCGCAAATG	
1862IRD800SET2R	GCGCCTCATCTACAGTCTTC	299
1862IRD800SET3F	GATGAGGGTATAAGGAGGTATC	
1862IRD800SET3R	CTTTGATGGTGATGTTTG	268
rafBIRD800irdf	CATTGGCGTCAGACATACTTAAG	
rafBIRD800irdr	GTCTAGGCGTTGAATGTG	222
rafBIRD800SET1F	AAGTGGGCTCGCTGGCG	
rafBIRD800SET1R	CACGCCACAGCAGCAGT	178
rafBIRD800SET2F	GCTGAAGCCGGAAGGAA	
rafBIRD800SET2R	AGCGTAACGGTACCTGC	172
rafAIRD800f	GACTCTCCTCAGCACGTTCTTC	
rafAIRD800r	GCCCTTAAGATCGCCGA	223
rafAPERP1	CTCACCGCCATCTATCTTG	
rafAPERP2	CGACGTATACGGAATGCATTG	
rafBPERP1	CACGCCACAGCAGCAGT	
rafBPERP2	AGCGTAACGGTACCTGC	
melAPERP1	GTTGGCTTTGTTGCTGGA	
melAPERP1	GCCAGCGCCGTCCCACA	
1862PERP1	CTAAATCAGTGCTGACATC	
1862PERP1	GCGCCTCATCTACAGTCTTC	

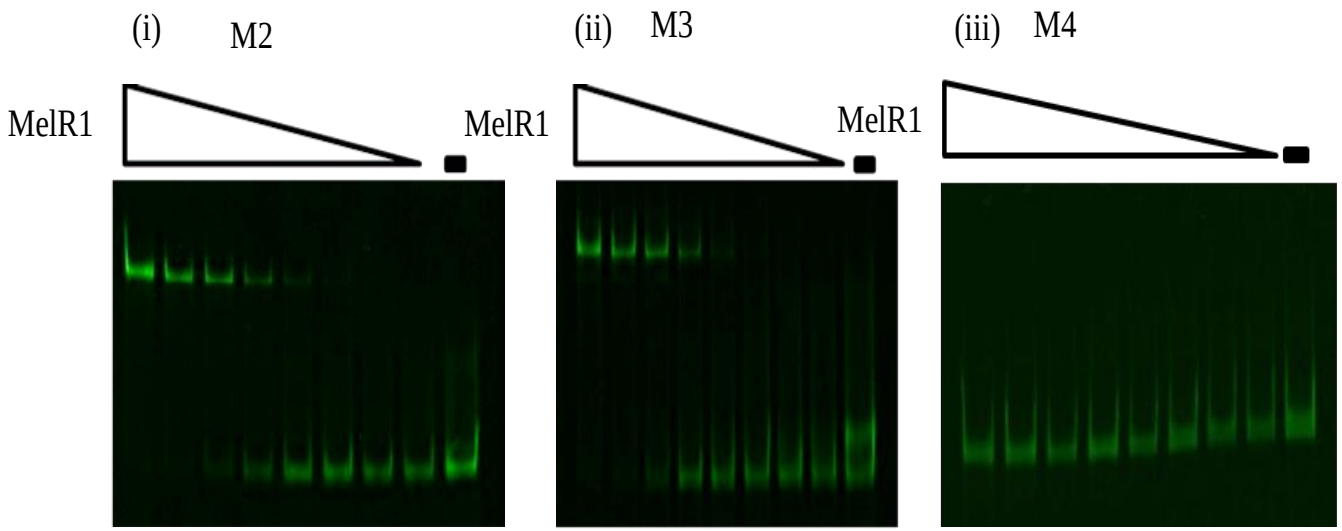

Figure S1 EMSAs showing MelR1 interaction with DNA fragments encompassing fragments (i) M2 (ii) M3 (iii) M4.

Table S3 IRD800 labelled annealed primers used to generate wild type sequence (wt) and also a series of mutated sequences (m) in relation to *melA* binding sequence

Wt	CTCATGCATAAGCGCTTAGCAAATACGCTAAGCCGAGATAGCCAAG
M1	CTCATGCATAAGCTATTAGCAAATACGCTAAGCCGAGATAGCCAAG
M2	CTCATGCATAAGCGCTTAGCAAATACGAGAAGCCGAGATAGCCAAG
M3	CTCATTAATAAGCGCTTAGCAAATACGCTAAGCCGAGATAGCCAAG
M4	CTCATGCATAAGCGCTTAGCAAATAATCTAAGCCGAGATAGCCAAG

Red lettering indicates the mutated base as compared to the WT sequence. All primers were annealed to reverse complementary sequence, forward primers indicated in table. (For details see materials and methods)

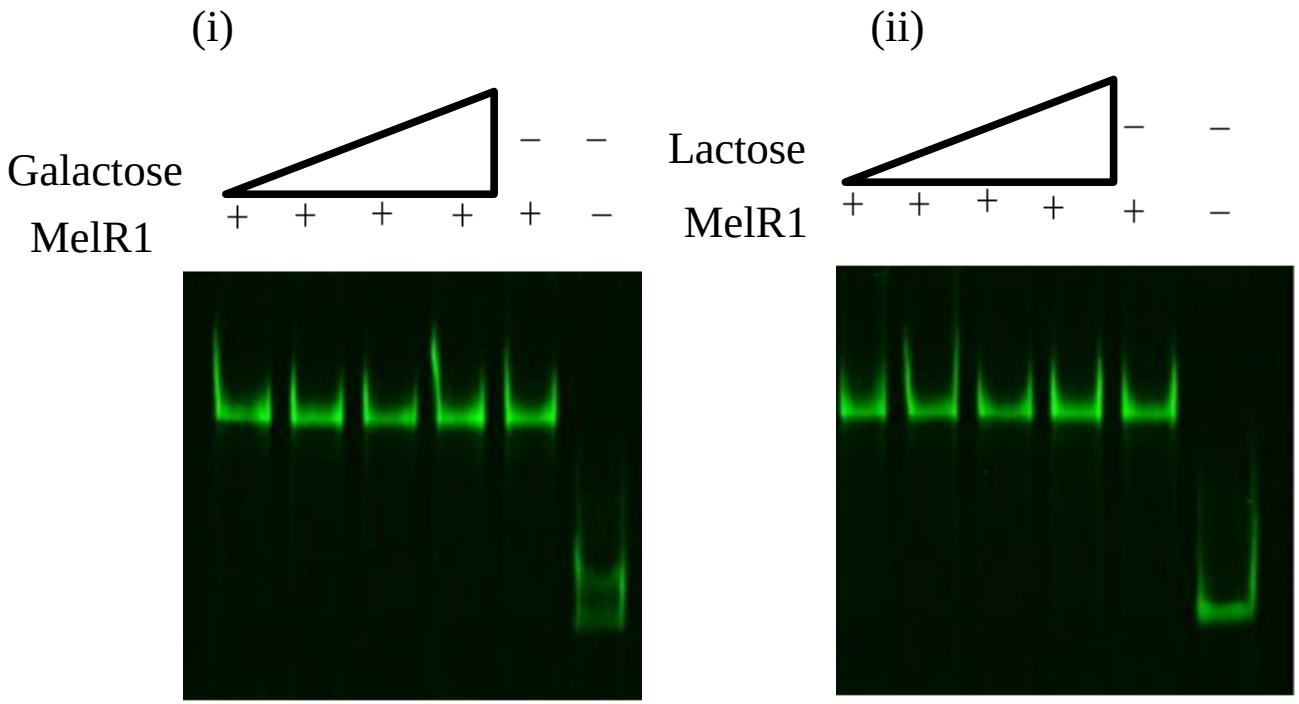

Figure S2 EMSAs showing MelR1 interaction with the DNA fragment M1 with the addition of (i) galactose and (ii) lactose at concentrations ranging from 2.5-20 mM.+ carbohydrate plus protein, + - protein no carbohydrate, -- no protein no sugar.

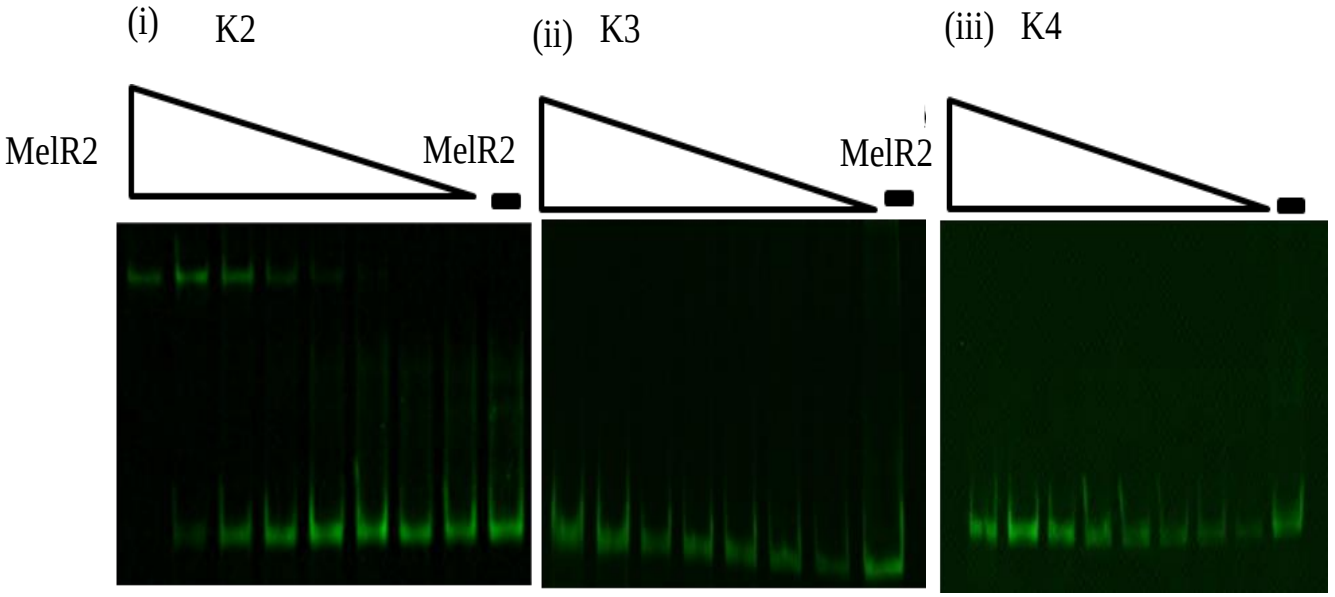

Figure S3 EMSAs showing MelR2 interaction with DNA fragments encompassing fragments (i) K2 (ii) K3 and (iii) K4.

Table S4 IRD800 labelled annealed primers used to generate wild type sequence (wt) and also a series of mutated sequences (m) in relation to *Bbr_1862* binding sequence.

Wt	TGACGGGTTCCGCAAAAAGATGTACGATGTGCGTAATCGATATCGCAAATG
M1	TGACGGGTTCCGCAAAAAGATGTACGCGGTGCGTAATCGATATCGCAAATG
M2	TGACGGGTTCCTAAAAAAGATGTACGATGTGCGTAATCGATATCGCAAATG
M3	TGACGGGTTCCGCAAAAAGATGTACGATGTGCGTACGCGATATCGCAAATG

Red lettering indicates the mutated base as compared to the WT sequence. All primers were annealed to reverse complementary sequence, forward primers indicated in table. (For details see materials and methods)

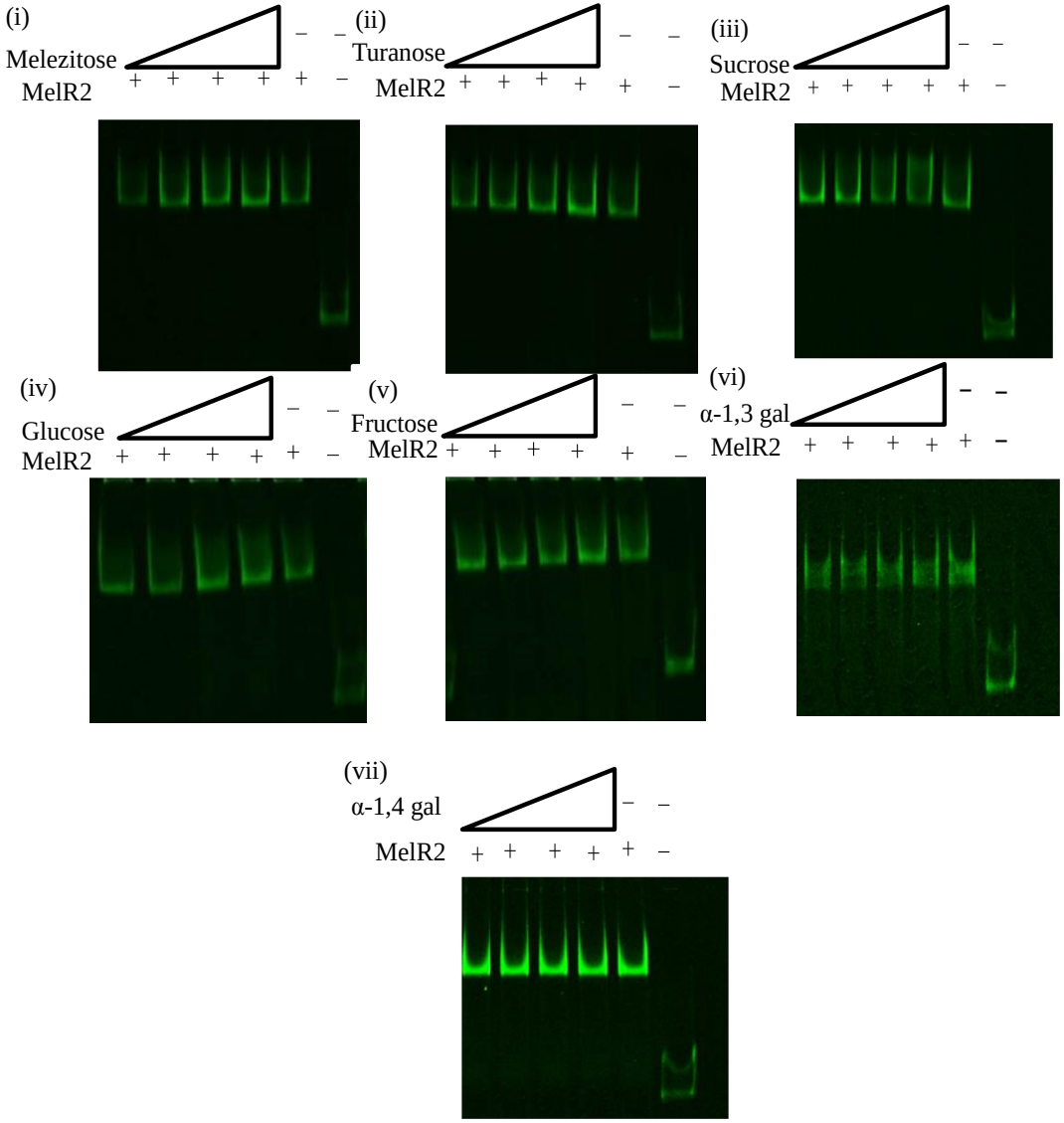

Figure S4 EMSAs showing MelR2 interaction with the DNA fragment K1 with the addition of (i) Melezitose (ii) Turanose (iii) Sucrose (iv) Glucose (v) fructose (vi) α-1,3 galactobiose (vii) α-1,4 galactobiose at concentrations ranging from 2.5-20 mM.+ carbohydrate plus protein, + - protein no carbohydrate, -- no protein no sugar.

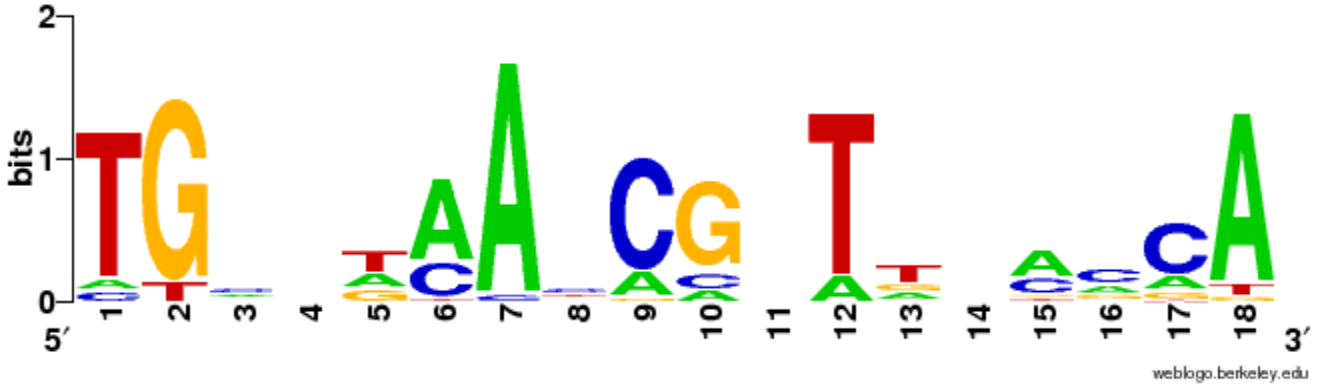

Figure S5 Weblogo of published LacI motifs present in *B. breve* UCC2003

Table S5. LacI motifs on genome of *B. breve* UCC2003

Locus tag	Gene	LacI binding site	E value	Closest LacI gene	gene distance
Bbr_0013	Bbr_0013	TGCGAAACCGCATGCACA	8.6	Bbr_0008	5
Bbr_0020	Bbr_0020	TGCTTAATCGCTTAAATG	68	Bbr_0019	1
Bbr_0021	Bbr_0021	TGATTCATCCGTACACCA	24	Bbr_0019	2
Bbr_0027	Bbr_0027	TGTGACAACGTTTGACCA	4.1	Bbr_0023	4
Bbr_0092	htpX	CGGTAAAGCGGTAAAACA	69	Bbr_0099	7
Bbr_0097	crcb1	TGCGGAATCGGACATCCA	1.40E+02	Bbr_0099	2
Bbr_0101	scrT	CGACTAACCGTTGGCCCA	44	Bbr_0102	1
Bbr_0106	cldE	TTTGGAAACGGTTCCAAA	92	Bbr_0105	1
Bbr_0111	agl3	TGCTGAACCCAATAACCA	21	Bbr_0112	1
Bbr_0113	Bbr_0113	TGCAAAACCGATTTCGCT	19	Bbr_0112	1
Bbr_0114	Bbr_0114	TGAATAACCAATATTCAA	54	Bbr_0112	2
Bbr_0116	malQ1	AGCAGAACAGGTTCAACA	72	Bbr_0112	4
Bbr_0122	Bbr_0122	TGCAGCACAGTTGACAGA	1.80E+02	Bbr_0122	0
Bbr_0285	lacZ2	TTCTGAAACGTTACAGAA	43	Bbr_0283	2
Bbr_0417	Bbr_0417	TGGTACACGGGTGTACCA	30	Bbr_0421	4
Bbr_0422	galA	TGATACACCGGTTGACCA	2.3	Bbr_0421	1
Bbr_0422	galA	TGATACACAGCTGTACCA	5.4	Bbr_0421	1
Bbr_0927	ilvE	TGAATCATAATTGCAACA	1.50E+02	Bbr_0934	7
Bbr_1419	rbsA1	TGATTAAACGTTAAATCA	1.9	Bbr_1420	1
Bbr_1427	trpF	TG-CATCACGATTTCGCA	1.50E+02	Bbr_1432	5
Bbr_1658	Bbr_1658	TGTCGAATCGGTTCGCGA	1.40E+02	Bbr_1659	1
Bbr_1742	Bbr_1742	TGGGTCATCCGTTTACCA	33	Bbr_1745	3
Bbr_1744	Bbr_1744	TGAATAAAAATTTTCGCA	67	Bbr_1745	1
Bbr_1827	Bbr_1827	TTAATAACCCAATAACCA	1.70E+02	Bbr_1831	4
Bbr_1842	aap6	AGCATAATCGCTGACGGA	1.90E+02	Bbr_1846	4
Bbr_1845	Bbr_1845	TGTAGAAACGCTGTAAAT	35	Bbr_1846	1
Bbr_1860	Bbr_1860	TGCATAAGCGCTTAGCAA	3.1	Bbr_1863	3
Bbr_1862	Bbr_1862	TGCGTAATCGATATCGCA	2.8	Bbr_1863	1

Table S6 IRD700 labelled primers used to generate annealed wild type sequence (wt) in combination with reverse complementary primer, only forward primer indicated, and also a series of mutated sequences (m) by PCR used in combination with RV in relation to RafR binding sequence (For details see materials and methods)

RV	GCGATGCGTATGCTCGGATC
WT	GCGCGGTATTTTGTGATTAACGCAATAAATAATGC
M1	TCGCGGTATTTTGTGATTAACGCAATAAATAATGC
M2	GAGCGGTATTTTGTGATTAACGCAATAAATAATGC
M3	GCTCGGTATTTTGTGATTAACGCAATAAATAATGC
M4	GCGAGGTATTTTGTGATTAACGCAATAAATAATGC
M5	GCGCTGTATTTTGTGATTAACGCAATAAATAATGC
M6	GCGCGTTATTTTGTGATTAACGCAATAAATAATGC
M7	GCGCGGGATTTTGTGATTAACGCAATAAATAATGC
M8	GCGCGGTCTTTTGTGATTAACGCAATAAATAATGC
M9	GCGCGGTAGTTTGTGATTAACGCAATAAATAATGC
M10	GCGCGGTATGTTGTGATTAACGCAATAAATAATGC
M11	GCGCGGTATTGTGTGATTAACGCAATAAATAATGC
M12	GCGCGGTATTTGGTGATTAACGCAATAAATAATGC
M13	GCGCGGTATTTTTTGATTAACGCAATAAATAATGC
M14	GCGCGGTATTTTGGGATTAACGCAATAAATAATGC
M15	GCGCGGTATTTTGTTTATTAACGCAATAAATAATGC
M16	GCGCGGTATTTTGTGCTTAACGCAATAAATAATGC
M17	GCGCGGTATTTTGTGAGTAACGCAATAAATAATGC
M18	GCGCGGTATTTTGTGATGAACGCAATAAATAATGC
M19	GCGCGGTATTTTGTGATTCACGCAATAAATAATGC
M20	GCGCGGTATTTTGTGATTACCGCAATAAATAATGC
M21	GCGCGGTATTTTGTGATTAAAGCAATAAATAATGC
M22	GCGCGGTATTTTGTGATTAACTCAATAAATAATGC
M23	GCGCGGTATTTTGTGATTAACGAAATAAATAATGC
M24	GCGCGGTATTTTGTGATTAACGCCATAAATAATGC
M25	GCGCGGTATTTTGTGATTAACGCACTAAATAATGC
M26	GCGCGGTATTTTGTGATTAACGCAAGAAATAATGC
M27	GCGCGGTATTTTGTGATTAACGCAATCAATAATGC
M28	GCGCGGTATTTTGTGATTAACGCAATACATAATGC
M29	GCGCGGTATTTTGTGATTAACGCAATAACTAATGC
M30	GCGCGGTATTTTGTGATTAACGCAATAAAGAATGC
M31	GCGCGGTATTTTGTGATTAACGCAATAAATCATGC
M32	GCGCGGTATTTTGTGATTAACGCAATAAATACTGC
M33	GCGCGGTATTTTGTGATTAACGCAATAAATAAGGC
M34	GCGCGGTATTTTGTGATTAACGCAATAAATAATTC
M35	GCGCGGTATTTTGTGATTAACGCAATAAATAATGA

Red lettering indicates the mutated base as compared to the WT sequence.

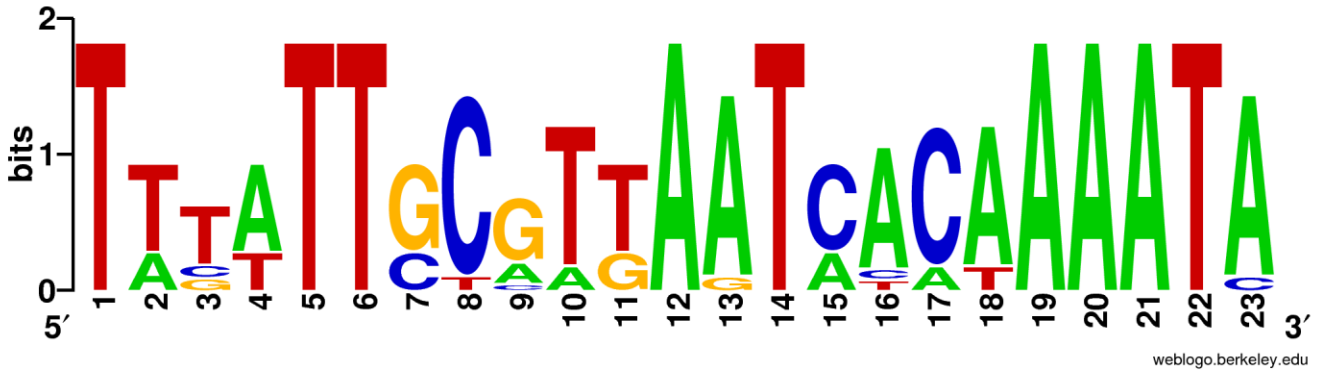

Figure S6 Analysis of sequenced bifidobacterial genomes, which all contain a homologue of the *B. breve* raf locus, reveals the presence of a single conserved RafR operator site in the *raf*B promoter region, whereas no other sites could be detected. Bases important for binding (as determined above) are conserved between bifidobacterial species

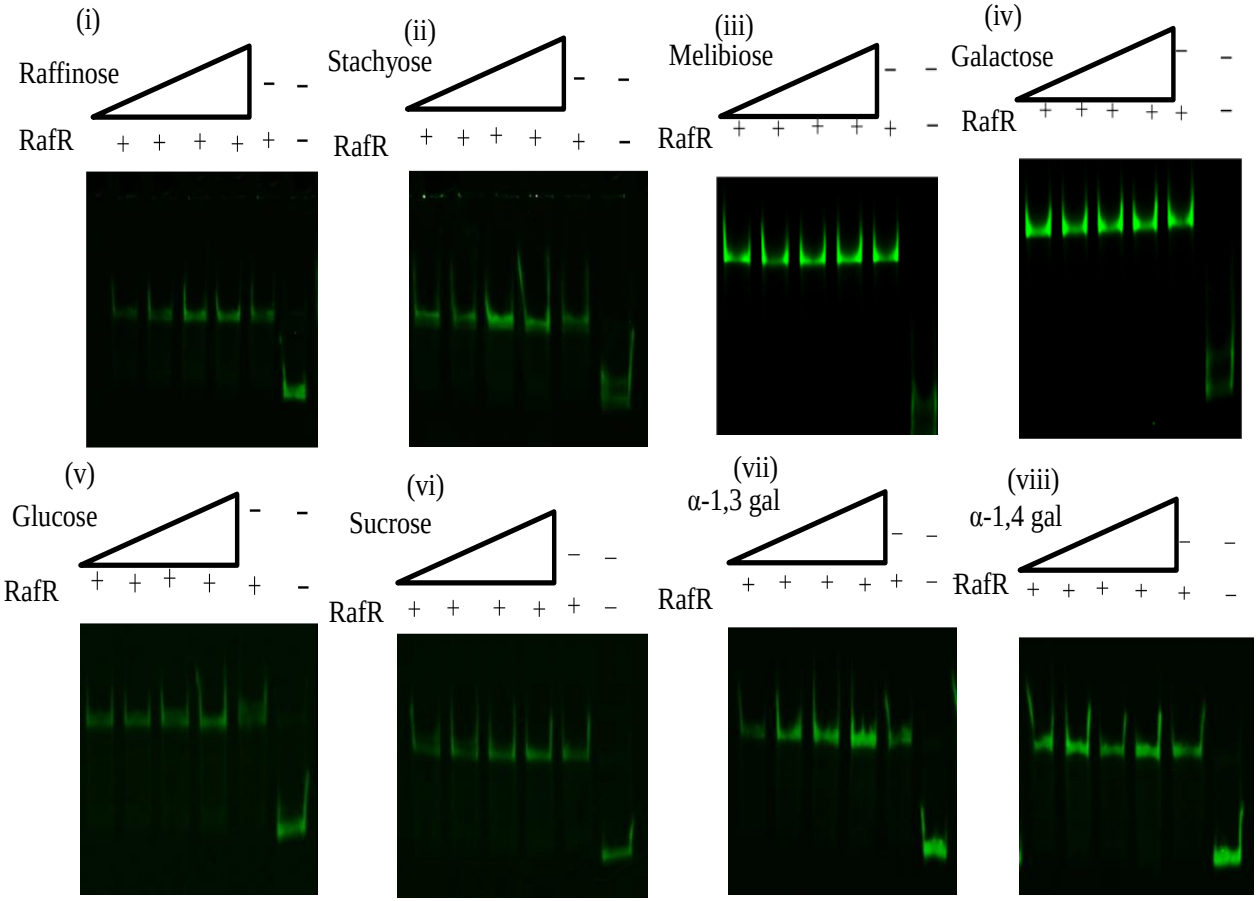

Figure S7 EMSAs showing RafR interaction with the DNA fragment R1 with the addition of (i) raffinose (ii) stachyose (iii) melibiose (iv) galactose (v) glucose (vi) sucrose (vii) α-1,3 galactobiose (viii) α-1,4 galactobiose at concentrations ranging from 2.5-20 mM.+ carbohydrate plus protein, + - protein no carbohydrate, -- no protein no sugar.

Table S7 Summary of EMSAs showing MelR1, MelR2 and RafR interactions.

Protein	Promoter region	Binding Sequence	Fragments bound	Inducing Carbohydrate
MelR1	*melA*	5’-TGCATAAGC><GCTTAGCAA-3’	M1, M2, M3	Melezitose
MelR2	*Bbr_1862*	5’-TGCGTAATC><GATATCGCA-3’	K1, K2	Unknown
RafR	*rafB*	5’-TTTATTGCGTT>A<ATCACAAAATA-3’	R1	Unknown