

Title	Functional carbohydrate binding modules identified in evolved Dits from siphophages infecting various Gram-positive bacteria
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Publication date	2018-09-11
Original Citation	Hayes, S., Vincentelli, R., Mahony, J., Nauta, A., Ramond, L., Lugli, G. A., Ventura, M., van Sinderen, D. and Cambillau, C. (2018) 'Functional carbohydrate binding modules identified in evolved Dits from siphophages infecting various Gram-positive bacteria', Molecular Microbiology. doi:10.1111/mmi.14124
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1111/mmi.14124
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Download date	2026-08-30 11:11:42
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Functional Carbohydrate Binding Modules Identified in evolved Dits from a Wide Range of Siphophages Infecting various Gram-positive Bacteria

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Supplementary Material

Figure S1. Sequence alignment of BppA and the four classes leader phage. a. Alignment of the five proteins. Note the good alignment of the junction domain. **b.** The BppA junction and CBM domain are presented as rainbow colored ribbons (Nt blue to Ct red).

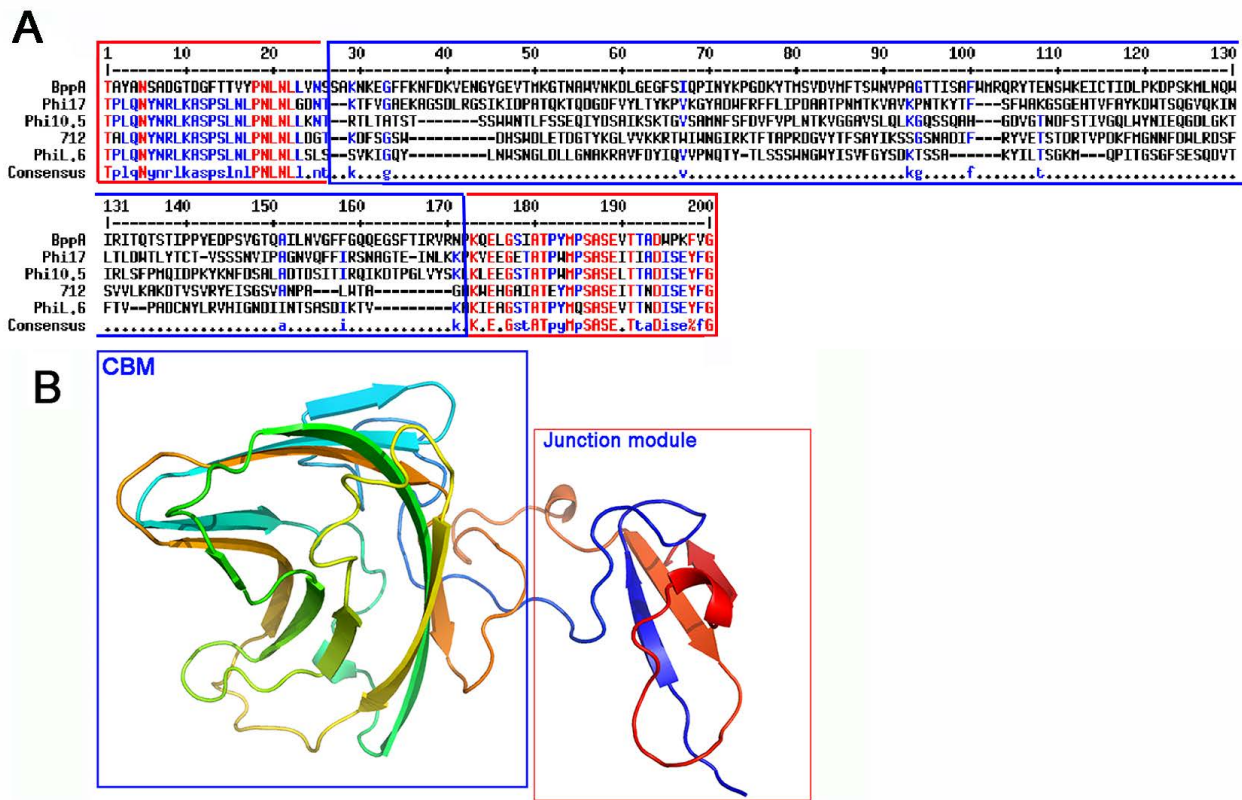
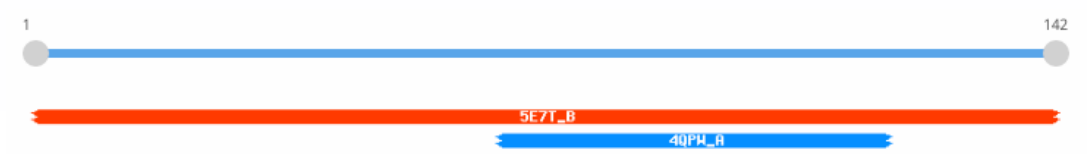


Figure S2. HHpred result of the CBM sequences of the Dit insertions which have been shortened by the removal of the small linker domain between the titin-like and the CBM domains. These residues were removed due to their high degree of similarity to BppA to ensure output would not be biased.

a Dit Class 1 shortened CBM



b Dit Class 3 shortened CBM

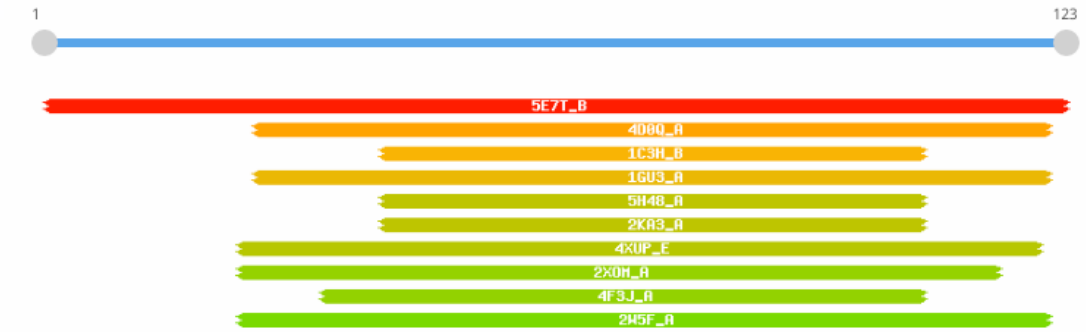


Figure S3. Binding of GFP-labelled “classical” Dits. a. Binding of the Dit of p2 to host strain NZ9000. Protein was added at a quantity of 100 μ g. b. Binding of the Dit of Phi4.2 to host strain *L. lactis* 4. Protein was added at a quantity of 100 μ g. Cells were visualized using differential interference contrast (DIC) microscopy (panels on the left), and fluorescent confocal microscopy (panels on the right) at the GFP excitement wavelength of 488 nm. Scale bars correspond to 10 μ m.

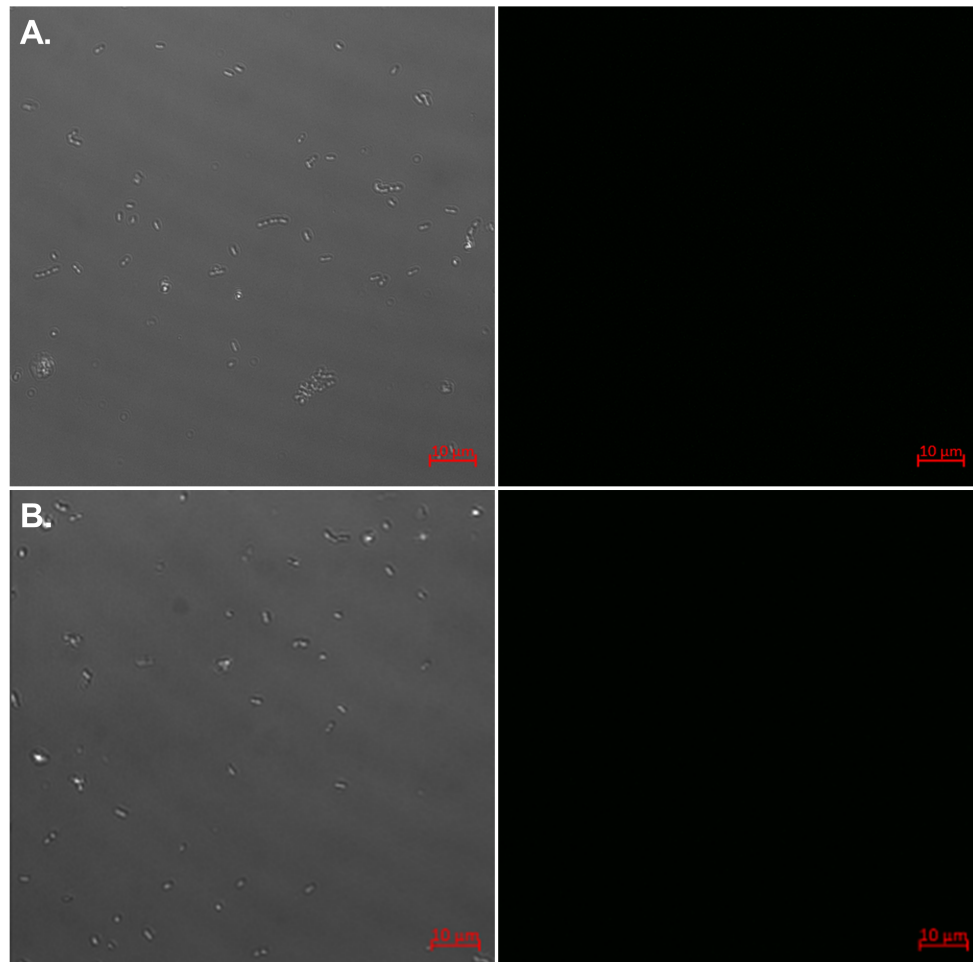


Figure S4. Host binding range assay of the GFP labelled Dit of phage Phi10.5 against 8 strains: four hosts sensitive to the indicated phages (panels a-d), and four non-host strains (panels e-h). a. Binding to host strain *L. lactis* 2 (CWPS Type B). b. Binding to host strain *L. lactis* 10 (CWPS Type B). c. Binding to host strain *L. lactis* 17 (CWPS Type B). d. Binding to host strain *L. lactis* 3 (CWPS Type B). e. Binding to host strain *L. lactis* C (CWPS Type A). f. Binding to host strain *L. lactis* 4 (CWPS Type C). g. Binding to host strain *L. lactis* 13 (CWPS Type C). h. Binding to host strain *L. lactis* A (CWPS Type C). In all cases, protein was added at a quantity of 5 μ g. Cells were visualized using differential interference contrast (DIC) microscopy (panels on the left), and fluorescent confocal microscopy (panels on the right) at the GFP excitement wavelength of 488 nm. Scale bars correspond to 10 μ m.

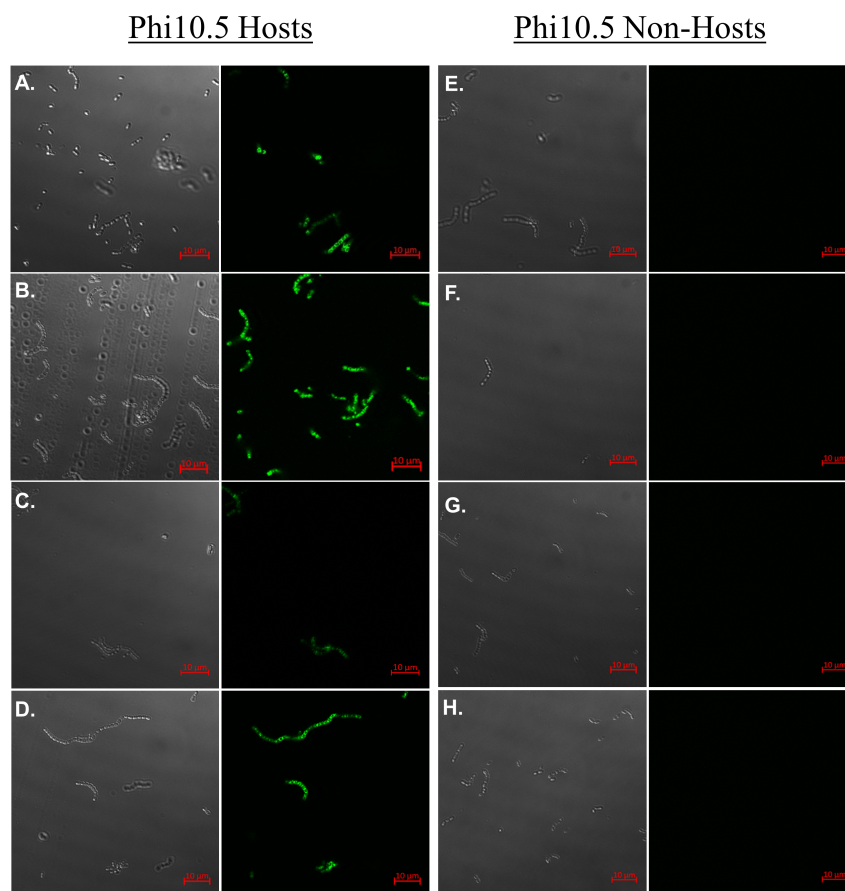


Figure S5. Host binding range assay of the GFP labelled Dlt of phage Phi2R14S against 8 strains: four hosts sensitive to the indicated indicated phages (panels a-d), and four non-host strains (panels e-h). a. Binding to host strain *L. lactis* 2 (CWPS Type B). b. Binding to host strain *L. lactis* 10 (CWPS Type B). c. Binding to host strain *L. lactis* 17 (CWPS Type B). d. Binding to host strain *L. lactis* 3 (CWPS Type B). e/ Binding to host strain *L. lactis* C (CWPS Type A). f. Binding to host strain *L. lactis* 4 (CWPS Type C). g. Binding to host strain *L. lactis* 13 (CWPS Type C). h. Binding to host strain *L. lactis* A (CWPS Type C). In all cases, protein was added at a quantity of 5 μ g. Cells were visualized using differential interference contrast (DIC) microscopy (panels on the left), and fluorescent confocal microscopy (panels on the right) at the GFP excitement wavelength of 488 nm. Scale bars correspond to 10 μ m

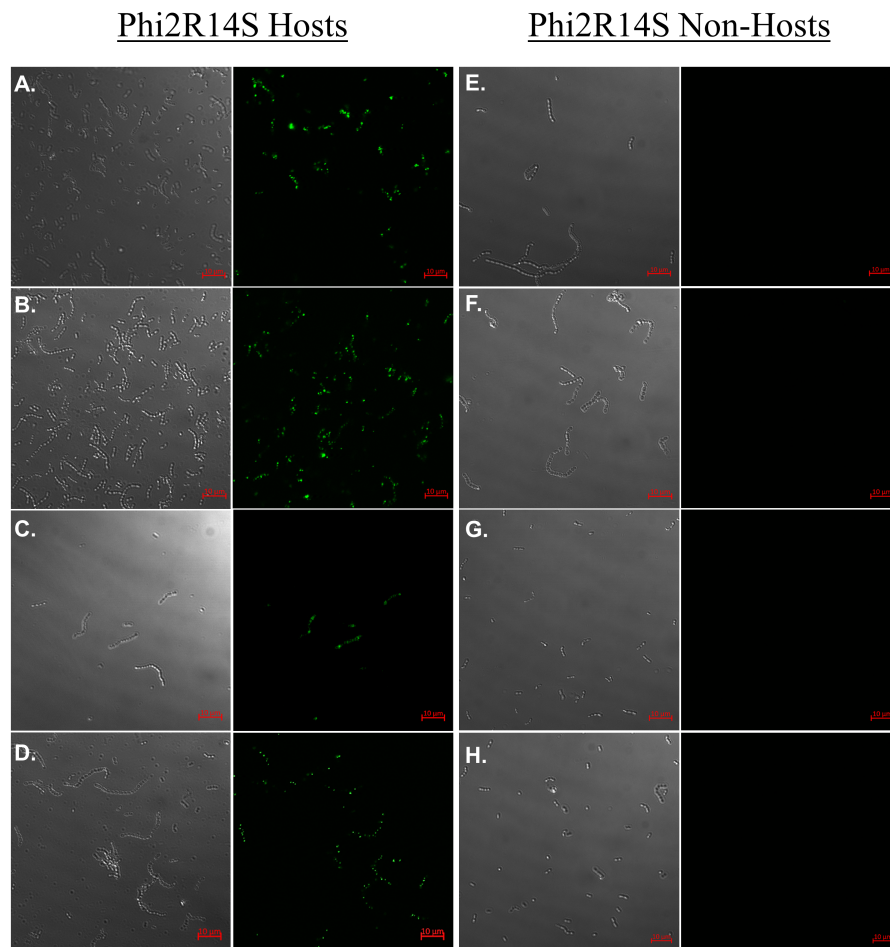


Figure S6. Host binding range assay of the GFP labelled Dit of phage PhiL.6 against 8 strains: four hosts sensitive to the indicated phages (panels a-d), and four non-host strains (panels e-h). a. Binding to host strain *L. lactis* 8 (CWPS Type C). b. Binding to host strain *L. lactis* 9 (CWPS Type C). c. Binding to host strain *L. lactis* L (CWPS Type unknown).unknown). d. Binding to host strain *L. lactis* M (CWPS Type C). e. Binding to host strain *L. lactis* 2 (CWPS Type B). f. Binding to host strain *L. lactis* 10 (CWPS Type B). g. Binding to host strain *L. lactis* 13 (CWPS Type C). h. Binding to host strain *L. lactis* C (CWPS Type A). In all cases, protein was added at a quantity of 15 μg . Cells were visualized using differential interference contrast (DIC) microscopy (panels on the left), and fluorescent confocal microscopy (panels on the right) at the GFP excitement wavelength of 488 nm. Scale bars correspond to 10 μm .

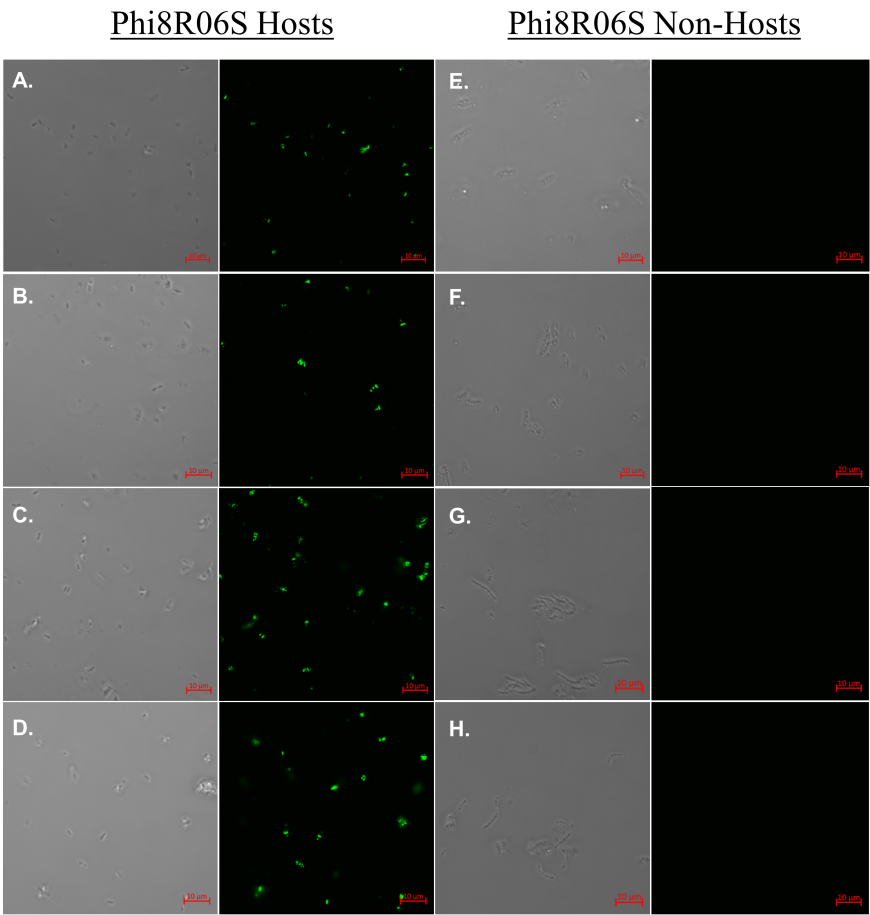


Figure S7. Host binding range assay of the GFP labelled Dlt of phage Phi8R06S against 8 strains: four hosts sensitive to the indicated phages (panels a-d), and four non-host strains (panels e-h). a. Binding to host strain *L. lactis* 8 (CWPS Type C). b. Binding to host strain *L. lactis* 9 (CWPS Type C). c. Binding to host strain *L. lactis* L (unknown CWPS Type). d. Binding to host strain *L. lactis* M (CWPS Type C). e. Binding to host strain *L. lactis* 2 (CWPS Type B). f. Binding to host strain *L. lactis* 10 (CWPS Type B). g. Binding to host strain *L. lactis* 13 (CWPS Type C). h. Binding to host strain *L. lactis* C (CWPS Type A). In all cases, protein was added at a quantity of 15 μ g. Cells were visualized using differential interference contrast (DIC) microscopy (panels on the left), and fluorescent confocal microscopy (panels on the right) at the GFP excitement wavelength of 488 nm. Scale bars correspond to 10 μ m.

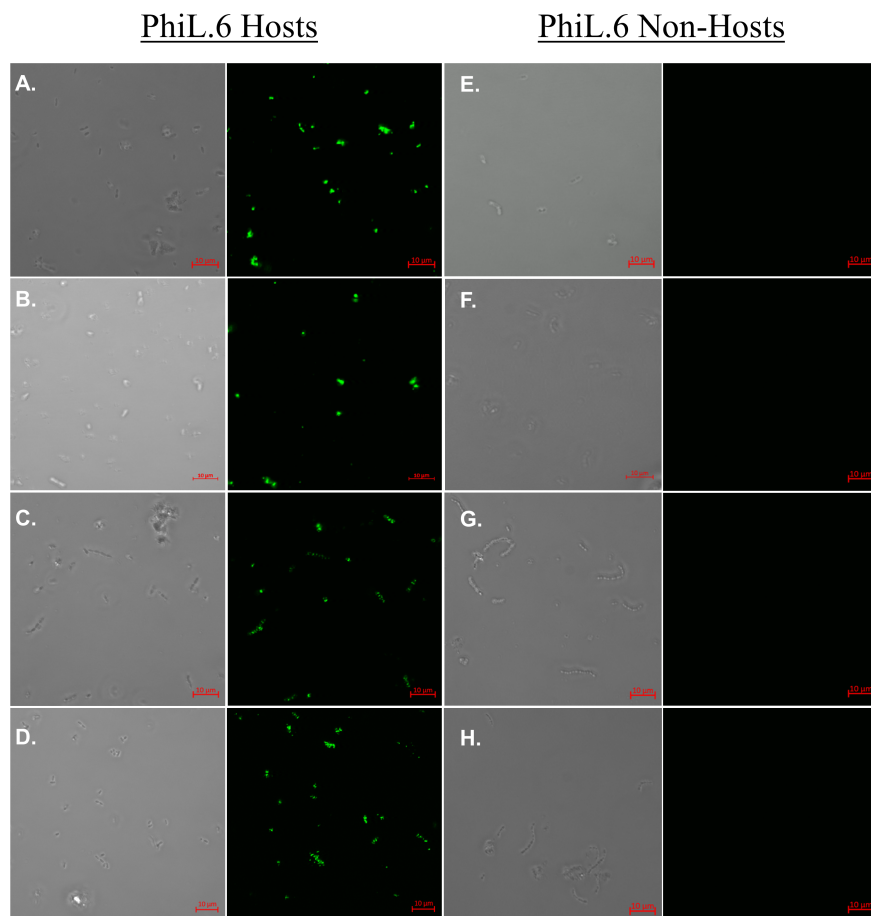


Table S1: Names, accession numbers and number of Dits amino-acids of the 936 phages used in this study.

Phage	Accession Nr	Dit	Phage	Accession Nr	Dit
PhiA.16	KP793102	298	Phi16W12L	KX379672	492
Phi19	KP793103	298	Phi16W23	KX346249	492
Phi4	KP793101	268	Phi17W11	KX346247	486
PhiB1127	KP793104	298	Phi17W12M	KX346248	486
Phi19.3	KP793105	280	PhiMW18L	KX379673	492
PhiD.18	KP793107	298	PhiMW18S	KX346250	492
PhiA1127	KP793106	298	340	KC182542	298
PhiLj	KP793133	298	645	KC182543	298
Phi5.12	KP793108	298	712	DQ227763	471
PhiC0139	KP793109	298	936	KC182544	298
Phi43	KP793110	292	ASCC191	JQ740787	298
Phi19.2	KP793111	280	ASCC273	JQ740788	298
PhiS0139	KP793134	298	ASCC281	JQ740789	298
Phi129	KP793112	298	ASCC284	JQ740790	298
PhiF.17	KP793113	298	ASCC287	JQ740791	298
Phi17	KP793114	491	ASCC310	JQ740792	298
Phi114	KP793115	298	ASCC324	JQ740793	298
Phi13.16	KP793116	298	ASCC337	JQ740794	298
PhiG	KP793117	298	ASCC356	JQ740795	298
PhiF0139	KP793118	298	ASCC358	JQ740796	298
Phi145	KM091444	491	ASCC365	JQ740797	298
PhiL.18	KP793120	491	ASCC368	JQ740798	298
Phi109	KP793121	491	ASCC395	JQ740799	298
PhiL.6	KP793122	466	ASCC397	JQ740800	298
Phi4.2	KP793123	310	ASCC406	JQ740801	298
Phi44	KP793124	298	ASCC454	JQ740802	298
Phi91127	KP793125	491	ASCC460	JQ740803	298
PhiM.5	KP793126	491	ASCC465	JQ740804	298
Phi16	KP793135	491	ASCC473	JQ740805	298
Phi40	KP793127	491	ASCC476	JQ740806	298
Phi10.5	KP793119	486	ASCC489	JQ740807	298
Phi93	KM091443	491	ASCC497	JQ740808	298
PhiM.16	KP793128	491	ASCC502	JQ740809	298
PhiJF1	KP793129	298	ASCC506	JQ740810	298
Phi15	KM091442	298	ASCC527	JQ740811	298
Phi155	KP793130	492	ASCC531	JQ740812	298
PhiE1127	KP793131	492	ASCC532	JQ740812	298
PhiM1127	KP793132	492	ASCC544	JQ740814	298
Phii0139	KX379665	298	bIBB29	EU221285	486
Phi2R14S	KX346236	486	bIL170	AF009630	298
Phi2R15M	KX346237	486	Caseus JM1	KC522412	298
Phi2R15S	KX346238	486	CB13	FJ848882	298
Phi2R15S2	KX346239	486	CB14	FJ848883	298
Phi2R06A	KX346240	486	CB19	FJ848884	298
Phi10W18	KX379664	486	CB20	FJ848885	298
Phi10W22S	KX379669	486	fd13	KC182545	298
Phi10W24	KX346245	486	jj50	DQ227764	298
Phi11W16L	KX346246	298	jm2	KC182546	298
Phi13W11L	KX379671	298	p2	GQ979703	298
Phi19W07F	KX379670	298	p008	DQ054536	298
Phi3R07S	KX346241	486	p272	KC182549	298
Phi3R16S	KX379666	486	p475	KC182550	298
Phi4R15L	KX379667	310	p680	KC182551	298
Phi4R16L	KX379668	310	p1132	KC182548	298
Phi8R06S	KX346242	466	phi7	KC182552	298
Phi6W06	KX346243	486	sk1	AF011378	298
Phi6W18L	KX346244	486	SL4	FJ848881	468
MP1		491			

Table S2. Bacterial strains used in this study.

<i>Bacterial strain</i>	<i>Source</i>
<i>Lactococcus lactis</i> strains A - T	[1]
<i>Lactococcus lactis</i> strains 1 - 20	[1]
NZ9000	[2]
IL1403	[3]
ASCC92	[4]
ASCC385	[4]
E8	[5]

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