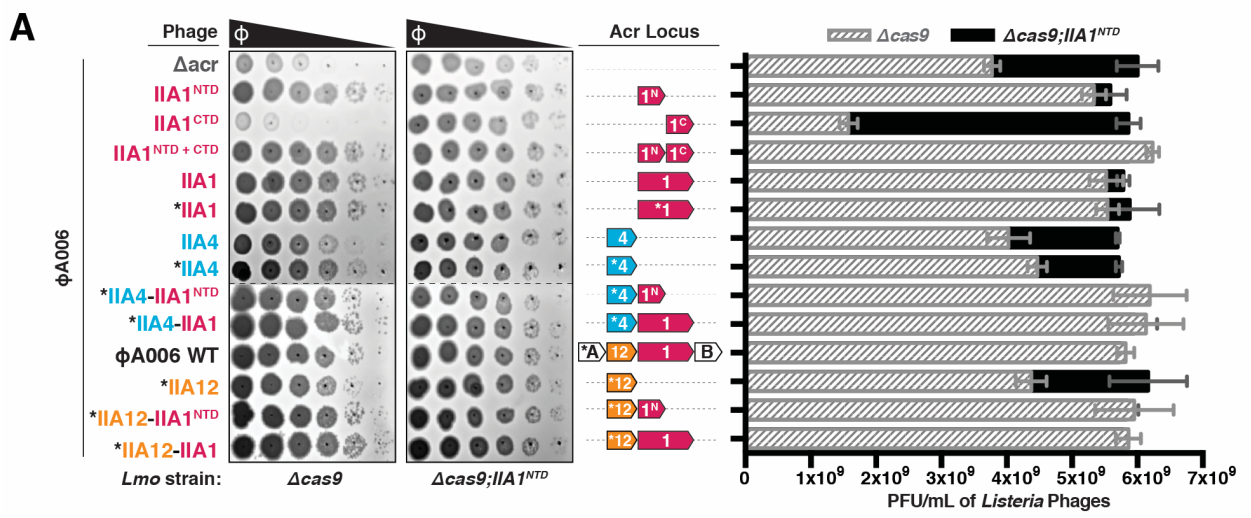

Figure S1. Optimal ΦA006 Phage Replication Requires AcrIIA1^{NTD}, Related to Figure 1
Left: Representative images of plaquing assays where the indicated *Listeria* phages were titrated in ten-fold serial dilutions (black spots) on lawns of *Lmo*10403s (gray background) lacking Cas9 (*Δcas9*) and encoding AcrIIA1^{NTD} (*Δcas9;IIA1^{NTD}*). Dashed lines indicate where intervening rows were removed for clarity. Right: Cas9-independent replication of isogenic ΦA006 phages containing distinct anti-CRISPRs. Asterisk (*) indicates genes that contain the strong RBS associated with orfA in WT ΦA006, whereas unmarked genes contain their native RBS. Plaque forming units (PFUs) were quantified on *Lmo*10403s lacking *cas9* (*Δcas9*, gray shaded bars) and expressing AcrIIA1^{NTD} (*Δcas9;IIA1^{NTD}*, black bars). Data are displayed as the mean PFU/mL of at least three biological replicates ± SD (error bars). Note that this figure contains the same subset of data displayed in Figure 1A.

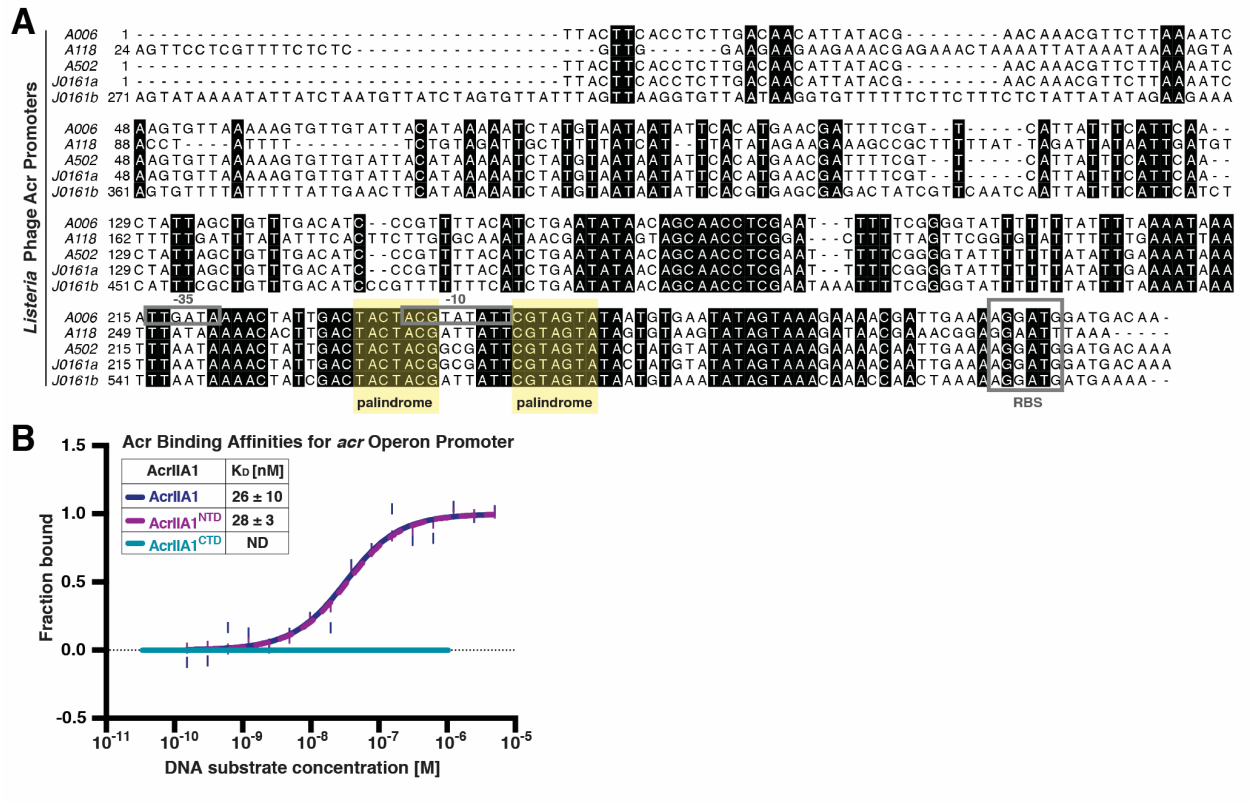

Figure S2. AcrIIA1^{NTD} Binds a Highly Conserved Palindromic Sequence in Acr Promoters, Related to Figure 2

(A) Alignment of the phage anti-CRISPR promoter nucleotide sequences denoting the -35 and -10 elements and ribosomal binding site (RBS) (gray boxes) and conserved palindromic sequence (yellow highlight). (B) Quantification of DNA binding abilities (K_D; boxed inset) of full-length AcrIIA1 and each domain (AcrIIA1^{NTD} and AcrIIA1^{CTD}) using microscale thermophoresis. Data shown are representative of three independent experiments. ND indicates no binding detected.

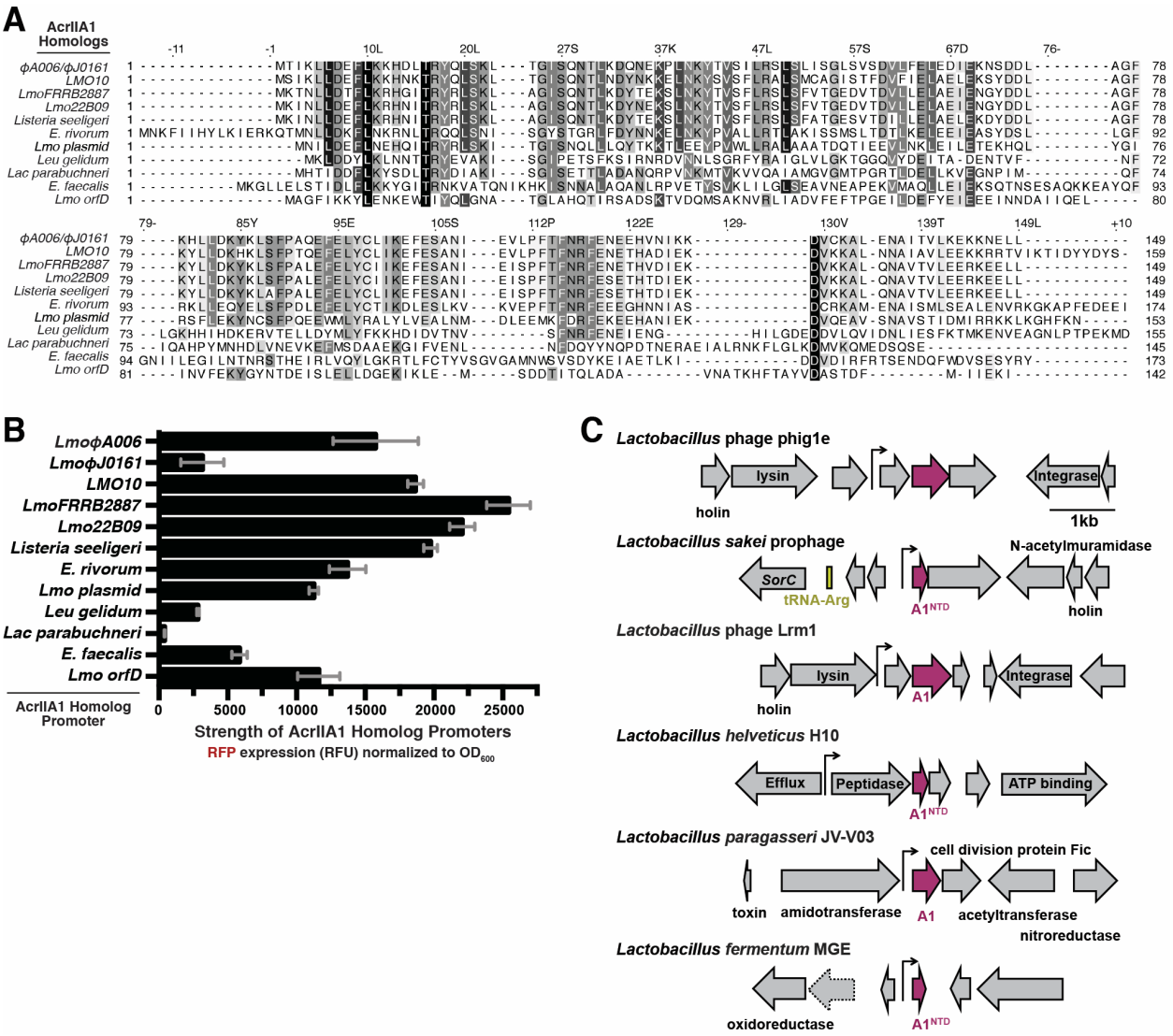

Figure S3. AcrIIA1 Homologs in Mobile Genetic Elements Across the *Firmicutes* Phylum Autoregulate their Cognate Promoters, Related to Figures 3, 4

(A) Alignment of AcrIIA1 homolog protein sequences. (B) Expression strength of the AcrIIA1 homolog promoters. Data are shown as the mean RFP expression (RFU normalized to OD_{600}) driven by each AcrIIA1 homolog promoter of three biological replicates ± SD (error bars). (C) Mobile genetic elements that possess an AcrIIA1 orthologue (red), which are either full-length or contain just the N-terminal domain ($A1^{NTD}$). Arrows indicate the region corresponding to the promoter that was experimentally tested for repression by host-associated $AcrIIA1^{NTD}$.

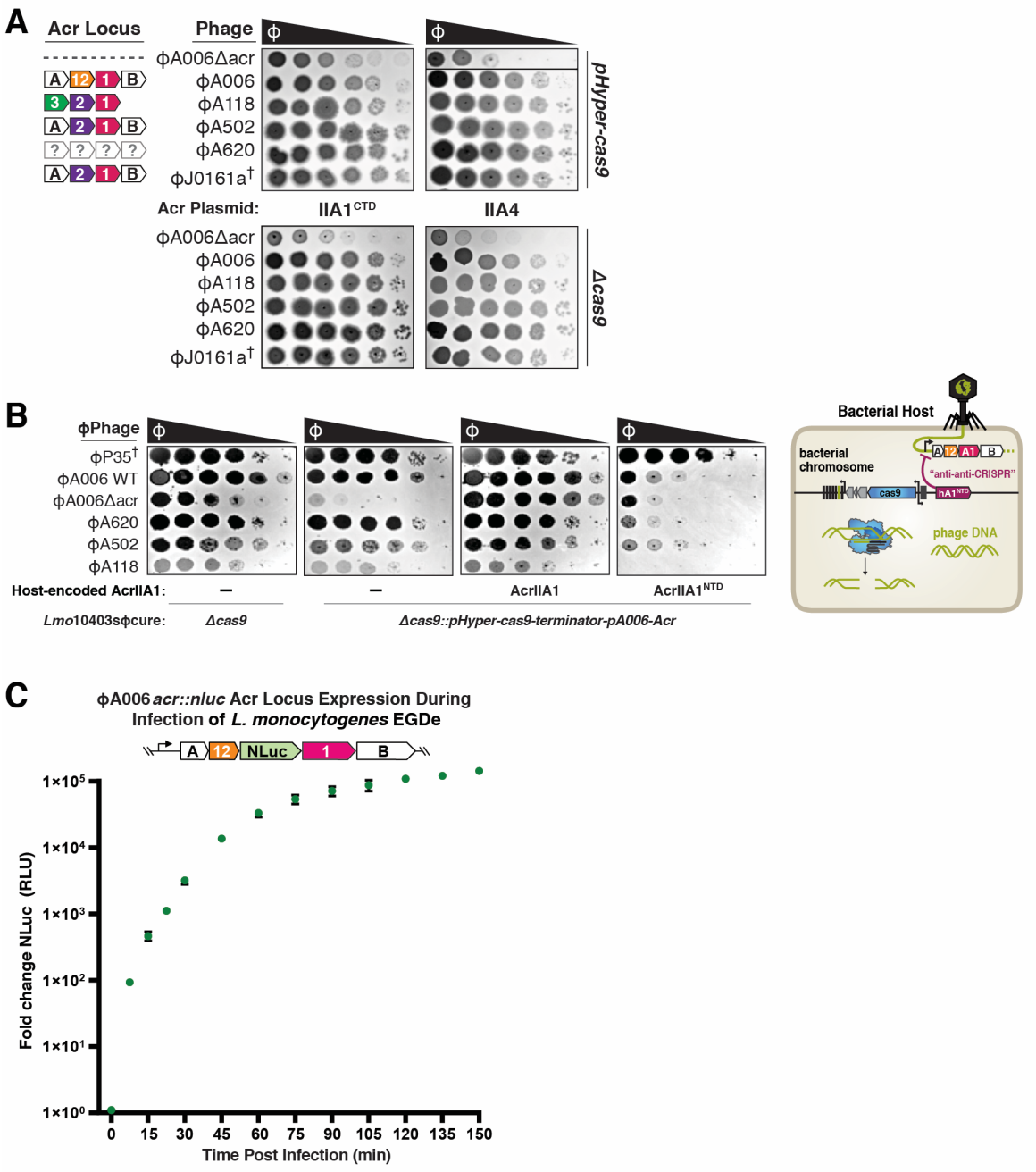

Figure S4. Bacterial expression of AcrIIA1^{NTD} blocks phage anti-CRISPR deployment, Related to Figure 4

(A) Plaquing assays where the indicated *L. monocytogenes* phages are titrated in ten-fold dilutions (black spots) on lawns of *L. monocytogenes* (gray background) expressing anti-CRISPRs from plasmids, LmoCas9 from a strong promoter (*pHyper-cas9*) or lacking Cas9 (*Δcas9*), and the natural CRISPR array containing spacers with complete or partial matches to the DNA of each phage. (†) Denotes the absence of a spacer targeting the ΦJ0161a phage. Representative pictures of 3 biological replicates are shown. Solid lines indicate where separate images are shown. (B) Left panels: Plaquing assays where wild-type *L. monocytogenes* phages are titrated in ten-fold dilutions (black spots) on lawns of *L. monocytogenes* (gray background) containing single-copy integrated constructs expressing AcrIIA1 or AcrIIA1^{NTD} from the ΦA006

anti-CRISPR promoter (pA006), LmoCas9 from a constitutive promoter (pHyper-Cas9), and the natural CRISPR array containing spacers with complete or partial matches to the DNA of each phage. (†) Denotes the absence of a spacer targeting the virulent phage ΦP35. Representative pictures of 3 biological replicates are shown. Right panel: Schematic of bacterial "anti-anti-CRISPR" activity where host-encoded AcrIIA1^{NTD} (hA1^{NTD}) blocks the expression of anti-CRISPRs from an infecting phage. (C) Nanoluciferase (NLuc) expression from the anti-CRISPR locus promoter of an ΦA006 reporter phage (ΦA006*acr::nluc*) during lytic infection of *L. monocytogenes* EGDe. Data are shown as the mean fold change in RLU (relative luminescence units) of three biological replicates ± SD (error bars).

Table S1. AcrIIA1 homolog protein accession numbers and associated promoter sequences, Related to Figures 3 and 4

Strains Containing AcrIIA1 Homologs	Designated Homolog Name	Protein Accession #	Associated Promoter Sequence (5' to 3')
Listeria monocytogenes J0161	LmoϕA006/ ϕJ0161	WP_003722518.1	tttacttcacctcttgacaacattatacgaacaaacgttcttaaaatcaagtgttaaaaagtgttgtatta cataaaaatctatgtaataatattcacatgaacgattttcgttcattatttcattcaactattagctgtttga catcccgttttacatctgaatataacagcaacctcgaatttttcggggtatttttttatattgaaaataaatt taataaaactattgactactacggcgattcgtagtatactatgtatatagtaaagaaaacaattgaaa aggatggatgacaaa
Listeria monocytogenes strain LMO10	LMO10	KUG37233.1	ttttgttgacgctttcacaaagacatgttattatatattcaagaacttaataagttctagcgctgtttcggc gcgttttaattacgcattgtgcaatgtaaatttctatgtatttaatttttatttagcacgaaaagaagctaca aattttaactacttactatgaaatgtaaggaaaaaaacatcagacttcggtttgatgttttttttactgtaaa aaaattaatccaataaaaaccattgactactacgattattcgtagtatataatatgtatatagtaaaggaa cgggaggaaaataca
Listeria monocytogenes strain FRRB 2887	LmoFRRB2887	WP_085696370.1	aataaaaagtaacctgtttttctatagattgcttttatcatatatatagaagaaagccgcttttattagatt ataattgatgtttttgatttatatttcactccctgtgcaaaataacgatatagtagcaacctcgaacttttttg ttcggggtatttttttgaaattaatttataaaaacacttgactactacgaatttacgtagtatactttaaatat agtaaagataacgaaacggaggaacttaaaa
Listeria monocytogenes isolate 22B09	Lmo22B09	WP_077316628.1	ttttatcagttatttttaaaaaaggttatctttcgtaaaacgcctatatgtagccgttttatagatagatagcc ttttttctttctgtttgaatcggtatattccagaaaagttttgccacgaatttgccacaaaatttgccgttgaa taattctttataaatatagtagtgcctcggacttttatggttcggggtatttttttgaaattaatttataaaaac actgactactacgaatttacgtagtatactttaaatatagtaaagataacgaaacggaggaacttaaaa
Listeria seeligeri FSL S4-171	Listeria seeligeri	EFS02359.1	ttgaaatgatgtacacgaacttgttcgctttagtagaaatagaccctcgcgacgaaaaaagatatta cttttccgacttaactcgtgatgaagtatttacaatgctgcaaaatatcaaaaataaataatgaatata gttgactaatacgaaaaatcgtagtatactgtgtatatagtaaagaaacgggaggagcttaaaa
Enterococcus rivorum strain LMG 258993	E. rivorum	WP_069698591.1	tgttcgtatttaggactataccgtaaaatttcgtacaactgatctggagataatcgcttattaaatgaga agattataataataaaaattgaaaacgttgatttaacagagttttcaaaaaaatataagaaaatatac cgtaaaatttcgttccactgatacgtggaccccaaaaattgaagtaaattgagcgaaactcttgatttc ttccgatttcggaagtataatagtgttataaggttgggataaggaaatagcacttccgcttaatcttaaa taaattaaaagaggatgaatgaa
Listeria monocytogenes CFSAN026587 plasmid	Lmo plasmid	WP_061665673.1	aacttacaatagtataggagcgttgctaatcattgctgtatgcttaaagaagtgcagatttaaaattag atatctttataacttttattaaattatagttgactattaaattataatttagtataataaaggtatagagataa gacataaaaatagaacaaatgaggtgcaatgac
Leuconostoc gelidum subsp. gasicomitatum KG16-1	Leu gelidum	CUR63869.1	tattattttccctctaaaataatagtacgtattaaacaagatgaactcttaatgttatttgccattagatata actgtaacacaatcgtaacattaatctattgcacactgcttaattaagcggtatacttaattcaaggtta aggaagaggtaaacgac
Lactobacillus parabuchneri strain FAM23166	Lac parabuchneri	WP_084975236.1	aacccctgtatagcataaaggttgcaatcctgccgagtgcataatcgcggtaaatcatcgattccgc atattcgttaatgtgatgcctccagtctctttagatgagagattggaggcatttttgcttttaaaaaccg atgttttatattgcatacttcgctgtaacgtagtaatattttaaaacatgaagttgcgacacacagttaac ttcgttatattttaacagtaaattcatggaggaaaaaca
Enterococcus faecalis strain plasmid Efsorialis-p2	E. faecalis	WP_002401838.1	ctaccataagttactgatagaaaagaaccaacagagtatgccttgttggttctttttctttgtccagttgtta ccaggtcagtagtaggacattcaaattgggcatacgtcatttgtgttaattttgagtacgctttaaattta catgtaatgaataaagtaatgggtattcgttttccactaactggccaaacagatagataggtgaaga acaaatttaacgcaaatggtaatgattgtgtttacatttaccttatatgtgatataatataagtgtaatca aagaagcctactcttgaaaattcaagaataggcaggtcgctaaacctctttgattataccatatatca aaggaagaaggaatgaaa
Listeria monocytogenes SLCC2540, serotype 3b	Lmo orfD	WP_012951927.1	acaaagaacatgcaaattatttaaaaagccgttcagctgcgcgatcttttattaagaaaaaagccac tttagaagacttggaagaactagaaattgcagtaaagcaaagaaaaactgaaataatttcattaga caatagccctgaatgaaaaatttcggggcatttttttattttttataatcaaatataattgactaatcaaata tatcgtgttatactatatatagtaaagaaacgggaggcgtacata
Lactobacillus delbrueckii strains	Lac delbrueckii	OOV09772.1	not applicable; AcrIIA1^{NTD} homolog in core bacterial genomes found next to Type I-E, I-C, or II-A CRISPR-Cas systems
Lactobacillus phage phig1e	ϕPhig1e	NP_695149.1	atatcctatcatgaatcgtatgtcatacctgtgctgggttaaaccagaacgggtatttttttgtgaaataa tacaataaaaactaaaagttaacacaatatgtgtttacaagtaatacaaaaggtgttaatatgtatttg tagaaaagaaaggaagtcttaaaa
Lactobacillus sakei prophage	Lac sakei	WP_076789011.1	tcttgaccactccttaaaaatcattatacgaacatacattctctttttgtcaaatttaatttaacctttttactta aatatattgacttatacctcaatatattgtattatataaatatggaaaggaggtgagagacatcggttct agggtagaaaaaaagaaccgactccgaaaagaaaagcgcgaagctaatgaatacagactgg ctgtcatatcattcataatatcgcttctagcatttctaaagagttggttctaagacacaggggcgaaag cccttgtttgtctactcataatacccatgtcatgctatgaaagacaaaagacacgcaactatggcacta tctatagctgctatcattctcagcgctttggcactacttgttaaatttctttaggaggctttttg
Lactobacillus phage Lrm1	ϕLrm1	YP_002117689.1	acaaatagtcctctgctcgctaacgcgggtggagggctttttttgttgtacttttttaaaaaataataccat ttatgattgtatgttagtaccgaatatggtactatataaacgtaaagaagaggaggagaacaaa
Lactobacillus helveticus prophage	Lac helveticus	WP_023060950.1	taaatttttcctattctcattcaaataattcttaccaatttattatattctttttgaatctgcttatggctgagatt tttgaaaaatactttaattaagaaaagcaatctattatactgaatacaatatgaaatttaaatgaaaag gtggcgcaagat
Lactobacillus paragresseri	Lac paragresseri	WP_003649108.1	aaatttaaataagttagtaaaatagcgaattagtgatcttgaaagtgactaatttgctatttttaattgaat gtttttcgaaaatatgagcttttgatttaaaataaggatagttaatatcagtgaggatactt
Lactobacillus brevis transposon	Lac brevis	WP_085769627.1	cctggtgataatccgcgtataccccgagtgagtagtctgaatcttggtaattactgagtaaccttaatttt tccgtcgttgaatatgttcctattcaaaacacccctagagatgacacttttattatttaaagtgtcaacc ctaaggggtattgtacgagaagaccagacattgttttattagtatgaaaagaggtgatgaacatggc ggaacgtccaatttttgtagaaatatgagcagcaagacgttctctaatttttattggtatagtagattgta aaattaatccgacgctgttcggacaaaaaagatcagcttcccttaaaatggtgtttaccacaaaccc atcttttaggagctgatctt
Lactobacillus fermentum MGE	Lac fermentum	WP_057195093.1	gatcgctaccagcccttcataaaacccgttcacccctacatagaaattatataatggcattagctcag gtgatagtctcttctatccttgagtcgtcccatcactatagggagaaagttatgactaatttttggatcgcc attctaattatggtaattatcattctcatccttgccattatccgttcactgctccaacttcgcctcaagtaatt gaaaggtcgccaaa