Supplementary Materials

for

YMC-2011, a Temperate Phage of *Streptococcus salivarius* 57.I (AEM03186-16R1)

Table S1. Properties of ORFs of YMC-2011

locus_tag	Direction[a]	Protein (aa/kDa)	Putative function	Similarity[b]	Best match[c]	Accession no.[d]
Ssal_phage00001	+	952/104.72	host specificity protein	Streptococcus phage Abc2, SP-Abc2_gp16	88 (733/953, 77%)	YP_003347425.1
Ssal_phage00002	+	681/74.91	minor structural protein	Streptococcus phage 7201, ORF39	81 (387/557, 69%)	NP_038340.1
Ssal_phage00003	+	89/9.79	hypothetical protein	Streptococcus phage Dp-1, orf56	68 (43/84, 51%)	YP_004306944.1
Ssal_phage00004	+	142/15.62	holin	Streptococcus phage Sfi19, orf141	84 (84/123, 68%)	NP_049940.1
Ssal_phage00006	+	73/8.03	holin	Streptococcus phage Abc2, orf21	87 (58/71, 82%)	YP_003347430.1
Ssal_phage00007	+	248/27.28	phage-associated cell wall hydrolase	Streptococcus phage phi3396, phi3396_56	70 (150/255, 59%)	YP_001039943.1
Ssal_phage00009	+	60/6.6	hypothetical protein	NA	NA	NA
Ssal_phage00010	-	353/38.83	integrase	Streptococcus phage O1205, ORF1	95 (321/353, 91%)	NP_695079.1
Ssal_phage00011	-	151/16.61	hypothetical protein	Streptococcus thermophilus LMD-9	55 (62/151, 41%)	YP_820257.1
Ssal_phage00012	-	121/13.31	CI-like repressor	Streptococcus phage Sfi21 , orf122 gp	93 (97/120, 81%)	NP_049992.1
Ssal_phage00013	-	114/12.54	CI-like repressor	Streptococcus phage Sfi21, orf127 gp	67 (50/96, 52%)	NP_049993.1
Ssal_phage00014	+	67/7.37	hypothetical protein	Streptococcus phage Abc2, orf25	93 (59/67, 88%)	YP_003347434.1
Ssal_phage00016	-	71/7.81	hypothetical protein	NA	NA	NA
Ssal_phage00017	+	244/26.84	phage antirepressor protein	Streptococcus phage Abc2, orf26	65 (116/205, 57%)	YP_003347435.1

Ssal_phage00018	+	37/4.07	hypothetical protein	NA	NA	NA
Ssal_phage00020	+	1993/10.23	hypothetical protein	Streptococcus phage Sfi21, orf80 (92) gp	95 (79/93, 85%)	NP_049996.1
Ssal_phage00022	+	51/5.61	hypothetical protein	Streptococcus phage O1205, ORF7	71 (26/45, 58%)	NP_695085.1
Ssal_phage00024	+	576/63.36	hypothetical protein	Streptococcus salivarius JIM8777	79 (418/586, 71%)	YP_006070072.1
Ssal_phage00025	+	106/11.66	hypothetical protein	Streptococcus salivarius JIM8777	84 (76/101, 75%)	YP_006070073.1
Ssal_phage00026	+	157/17.27	hypothetical protein	Streptococcus phage ALQ13.2, orf33	78 (93/157, 59%)	YP_003344879.1
Ssal_phage00027	+	233/25.63	hypothetical protein	Streptococcus phage 858, orf36	99 (227/233, 97%)	YP_001686830
Ssal_phage00028	+	451/49.61	helicase	Streptococcus phage 858, orf37	92 (417/451, 92%)	YP_001686831.1
Ssal_phage00029	+	157/17.27	phage protein	Streptococcus phage 858, orf38	94 (142/157, 90%)	YP_001686832.1
Ssal_phage00030	+	272/29.92	phage protein	Streptococcus phage 858, orf39	91 (237/271, 87%)	YP_001686833.1
Ssal_phage00031	+	519/57.09	primase	Streptococcus phage 858, orf40	93 (466/514, 91%)	YP_001686834.1
Ssal_phage00032	+	107/11.77	phage protein	Streptococcus phage 858, orf41	96 (96/103, 93%)	YP_001686835.1
Ssal_phage00033	+	72/7.92	hypothetical protein	Streptococcus phage DT1,Orf39	77 (53/71, 75%)	NP_049427.1
Ssal_phage00034	+	60/6.6	hypothetical protein	NA	NA	NA
Ssal_phage00035	+	61/6.71	hypothetical protein	NA	NA	NA
Ssal_phage00036	+	52/5.72	hypothetical protein	Streptococcus phage Sfi19, orf51	79 (38/52, 73%)	NP_049960.1
Ssal_phage00037	+	96/10.56	hypothetical protein	Streptococcus phage O1205, ORF21	89 (71/83, 86%)	NP_695099.1
Ssal_phage00038	+	115/12.65	hypothetical protein	NA	NA	NA
Ssal_phage00039	+	235/25.85	hypothetical protein	Streptococcus phage Sfi19, orf235	60 (125/243, 51%)	NP_049965.1
Ssal_phage00040	+	132/14.52	hypothetical protein	Streptococcus phage 7201, ORF19	92 (116/132, 88%)	NP_038320.1
Ssal_phage00041	+	144/15.84	HNH endonuclease	Streptococcus phage Abc2, orf48	96 (133/144, 92%)	YP_003347457.1

Ssal_phage00042	+	193/21.23	terminase small subunit	Streptococcus phage Sfi19, orf161	84 (118/152, 78%)	NP_049925.1
Ssal_phage00043	-	46/5.06	hypothetical protein	NA	NA	NA
Ssal_phage00045	+	292/32.12	hypothetical protein	NA	NA	NA
Ssal_phage00046	+	63/6.93	hypothetical protein	NA	NA	NA
Ssal_phage00047	+	229/25.19	terminase small subunit	Streptococcus phage DT1,orf3	82 (148/215, 69%)	NP_049391.1
Ssal_phage00048	+	390/42.9	terminase large subunit	Streptococcus phage Abc2, orf2	91 (325/391, 83%)	YP_003347411.1
Ssal_phage00049	+	59/6.49	head-tail joining protein	Streptococcus phage DT1, orf5	98 (52/59, 88%)	NP_049393.1
Ssal_phage00050	+	383/42.13	portal protein	Streptococcus phage Abc2, orf4	96 (345/386, 89%)	YP_003347413.1
Ssal_phage00051	+	222/24.42	scaffold protein	Streptococcus phage Abc2, orf5	94 (193/222, 87%)	YP_003347414.1
Ssal_phage00052	+	396/43.56	major head protein	Streptococcus phage Sfi19, orf397	95 (357/392, 91%)	NP_049929.1
Ssal_phage00053	+	104/11.44	DNA packaging protein	Streptococcus phage Sfi19, orf104	96 (95/104, 91%)	NP_049930.1
Ssal_phage00054	+	116/12.76	capsid-tail joining protein	Streptococcus phage Abc2, orf8	94 (104/116, 90%)	YP_003347417.1
Ssal_phage00055	+	142/15.62	tail component protein	Streptococcus phage Sfi19, orf153	96 (125/139, 90%)	NP_049932.1
Ssal_phage00056	+	123/13.53	tail component protein	Streptococcus phage DT1, orf12	97 (112/123, 91%)	NP_049400.1
Ssal_phage00057	+	205/22.55	major tail protein	Streptococcus phage Abc2, orf11	92 (163/203, 80%)	YP_003347420.1

Ssal_phage00058	+	118/12.98	tail component protein	Streptococcus phage DT1, orf14	92 (96/118, 81%)	NP_049402.1
Ssal_phage00059	+	47/5.17	hypothetical protein	NA	NA	NA
Ssal_phage00061	+	773/85.03	minor tail protein	Streptococcus phage Sfi19, orf1626	90 (665/791, 84%)	NP_049935.1
Ssal_phage00063	+	890/97.9	minor tail protein	Streptococcus phage Sfi21, orf1560	85 (682/889, 77%)	NP_049978.1
Ssal_phage00064	+	518/56.98	tail component protein	Streptococcus phage Abc2, orf15	87 (404/523, 77%)	YP_003347424.1

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[a] The relative orientation of ORFs: +, forward; -, reverse.

[b] The target to which the ORF shares the highest homology. NA, not available.

[c] % similarity. Numbers in parentheses are the length of the alignment/target size and % identity.

[d] GenBank number of the locus listed under Similarity.

Fig. S1A

A

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                        •         •         •         •         •         •
ALQ13.2   32341-------------------------------------------TGAAAATAAAGGGTTTC
O1205      8875-------CCGCAGGTTATTGCAACAAGTAACCGTAAAACCCATTGAAAATAAAGGGTTTC
DT1       30059-------CCGCAGGTTATTGCAACAAGTAACCGTAAAACCCATTGGAAATAAAGGGTTTC
2972      32216----TACCTACAGACACCAA-GAGCGGTGTCCTCGAAAGTCCTTGATAGAAAAGGAATTG
YMC-2011  19816--GGTTACCGTAAAAAATGACATACGGTAACCCTCACAAACCCTTTAATGATACCGTTTT
                                                          *   *  * *   **

                        •         •         •         •         •         •
               GGTTGCTTTAGTTACTTAGTTACTACTTTTAAATATATTTATAA-TAAATAAATAAATAA
               GGTTGCTTTAGTTACTTAGTTACTACTTTTAAATATATTTATAAATAAATAAATAAATAA
               GGTTGCTTTAGTTACTTAGTTACTATATATATATATATTTATAAATAAATAAATAAATAA
               ACACGCTTTAGACACTAAGATACTACTTTTATATATATTTAAAA-TAAATAAATAAATAA
               TCTCTACTTAGTTACCTTGTTACCTTATTTATATATTGAAATAA-TAAATAAATAAATAA
                      ****  **   * ***    * ** ****    * ** ***************

                        •         •         •         •         •         •
               ATATA-TATATATATAGAGAGAGCCATCAAAAAAACGTGTAACTAAGTAACTAAAG--TG
               ATAAA-TATATATAGAGAGAGA--C-TTAAAAAAACGTGTAACTAAGTAACTAAAG--TG
               ATATA-TATATATAGAGAGAGA--CACTTAAAAAACGTGTAACTAAGTAACTAAAG--TG
               ATATA-TATATAGAGAGAGAG-----TCAAAAAATAAGGTGCCTCGGTGCCCAAAA--GT
               ATAAAGTATATATAAGTAGAGC--TTACAACGGTAACGGTAACGAATTAGCAAAAAAACA
               ***** ****** *   ****        *        **  *    *  * ***

                        •         •         •         •         •         •
               GCCAGAAACCTTGATATATAA-GGGTTTGCGGCGGTTACGAGTAAAAGTAACTGTTACTG
               GCCAGAAACCTTGATATATAAGGGGTTTGCGGTGGTTACGAGTAAAAGTAACTGTTACTG
               GCCAGAAACCTTTATATATAA-GGGTTTTCGGCGGTTACGAGTAAAAGTAACTGTTACTG
               GTCAAAAACCCTTATATATCAAGGGTTTTCG-TGGGCACTGCTAAGAGTGTCTTGGATGG
               GTCAAAACCGTTGATAT-CATTGGGTTTTAGACGGTTACTGTTCTAAA-AATAAGAAAGG
               * ** ** *  * ****     ******  *  **  **   *   *         *  *

                        •         •         •         •
               TAATCGAGTAACAATTAGGAGAAAAAATACTTAGAGATTAA-32574
               TAATCGAGTAACAAA-AGGAGAAAAAA-9127
               TAATCGAGTAACAAA-AGGATAAAAAA-30311
               TGACCGGTGACAAATTAGGAGATAT-32465
               TAACTATTTA-20057
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Fig. S1B

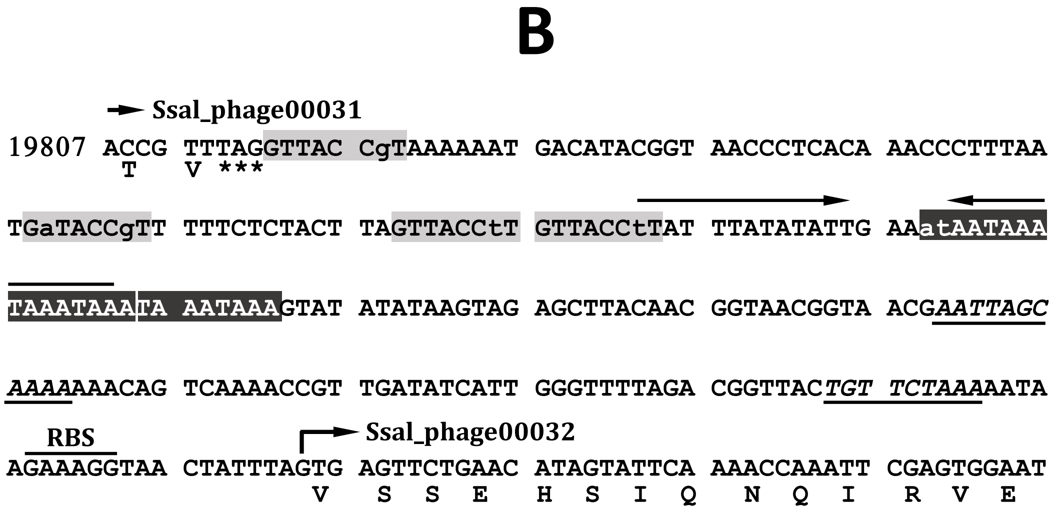

FIG. S1. Nucleotide sequence analysis of the putative *ori* of YMC-2011. (A) Sequence alignment of the putative *ori* sequences of phages ALQ13.2, O1205, DT1, 2972 and YMC-2011. The numbers indicate position of each sequence on the phage genome. Identical bases are indicated by asterisks below the alignment. Dots above the sequence denote 10-base divisions. (B) The putative *ori* of YMC-2011 is located in the intergenic region between Ssal_phage00031 and Ssal_phage00032. The location of the stop codon of Ssal_phage00031 is indicated by asterisks. The start codon and RBS of Ssal_phage00032 are indicated by a bent arrow and an overline, respectively. The deduced aa sequence of the ORFs is listed below the nucleotide sequence. The putative DnaA boxes are underlined and italicized. The two sets of direct repeats are shaded in light grey and black, respectively. Nucleotides that differ between repeat sequences are in lower case. The inverted repeats are indicated by horizontal arrows above the sequence.

Fig. S2

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                  •         •         •         •         •
7201     -GCCCCCTATATCGAGCCTAGGAGAGCCGCCACAAGGTGTCTTCTTACATCACGCG
Abc2     ---CCCTATA-TCGAGCCTAGGAGAGCCACGACAAGGTGTCTTCTCTTGTCACGCG
DT1      -CCCCCTATA-TCGAGCCTAGGAGAGCCACCACAAGGTGTCTTCTCTTGTCACGCG
Sfi19    -CGCCCTTCCTAGAGGCCTAGGAGAGCCACGACAAGGTGTTTTCTTACGTCACGCA
Sfi21    -GCCCCTATAATCTAAAATAGGGGAGCCGCCACAAGGTGTCTTGTCTTGTCGCACG
YMC-2011 -CGCCCCCCTCTCGTGCCAAGGAGAGCCGCCACAAGGTGTTCTTTTATGTCGGATA
            ***             *** ***** * *********  * *   ***

                  •         •         •
         C-AATTTTTGAGAATTTTTAAGTGGTGTCTATACCA
         C-AACTTTTGAGGATTTTTAAAGGGTGTCTATACCA
         C-AACTTTTGAGGATTTTTAAAGGGTGTCTAGACCA
         C-AATTTTTGAGGATTT-------------------
         C-AACTTTTGAGGATTTTTAAAGGGTGTCTATACCA
         CCAATTTTTCAGATTTTTAAAGG-------------
         * ** **** **  ***
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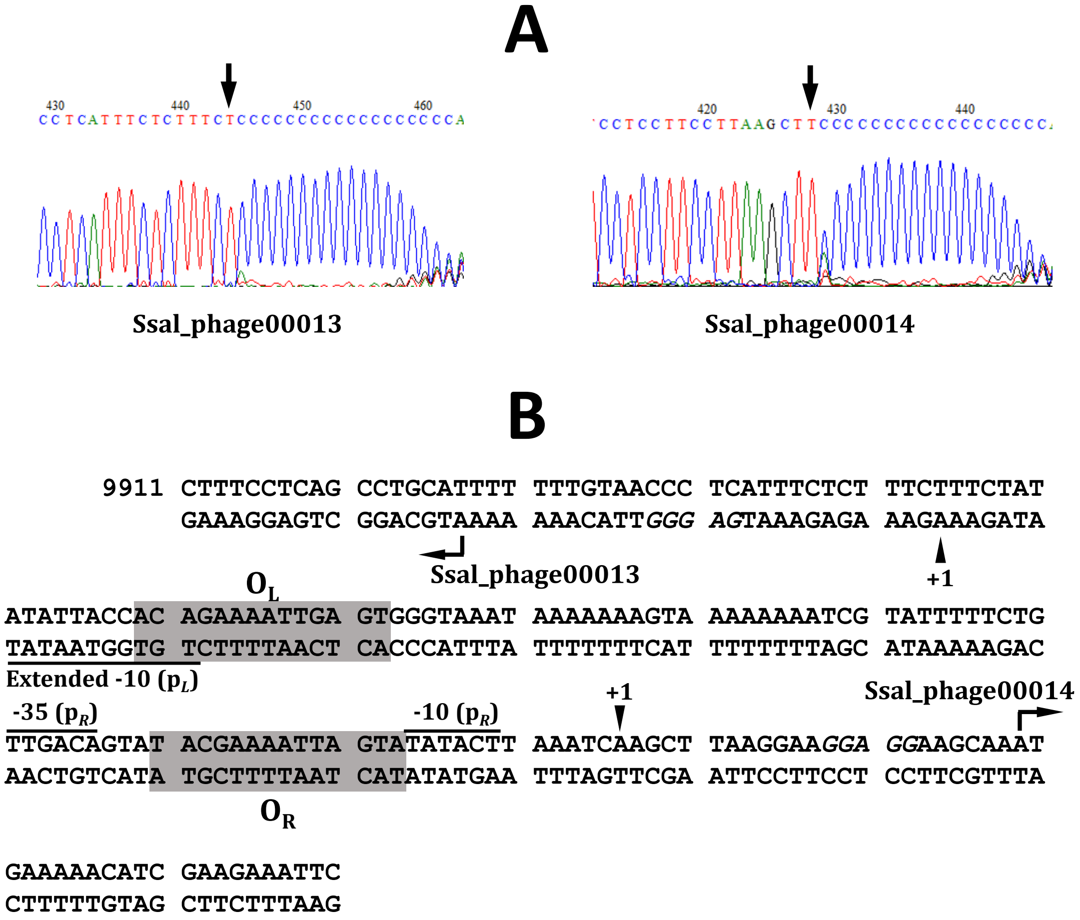
FIG. S2. Sequence alignment of the *cos* site. The consensus sequences of the *cos* site from *S. thermophilus* phages and phage YMC-2011 were analyzed. Identical bases are indicated by asterisks below the alignment. The predicted *cos* site is shaded.

Fig. S3



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9911 CTTTCCTCAG CCTGCATTTT TTTGTAACCC TCATTTCTCT TTCTTTCTAT
     GAAAGGAGTC GGACGTAAAA AAACATTGGG AGTAAAGAGA AAGAAAGATA

ATATTACCAC AGAAAATTGA GTGGGTAAAT AAAAAAAGTA AAAAAAATCG TATTTTTCTG
TATAATGGTG TCTTTTAACT CACCCATTTA TTTTTTTCAT TTTTTTTAGC ATAAAAAGAC

TTGACAGTAT ACGAAAATTA GTATATACTT AAATCAAGCT TAAGGAAGGA GGAAGCAAAT
AACTGTCATA TGCTTTTAAT CATATATGAA TTTAGTTCGA ATTCCTTCCT CCTTCGTTTA

GAAAAACATC GAAGAAATTC
CTTTTTGTAG CTTCTTTAAG
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FIG. S3. Identification and sequence analysis of the promoters of Ssal_phage00013 and Ssal_phage00014. (A) The transcription initiation sites of Ssal_phage00013 and Ssal_phage00014 were determined by 5' RACE. Sequencing analysis of the final PCR products described in Materials and Methods is shown. The transcription initiation site is indicated by a vertical arrow. (B) The intergenic region of Ssal_phage00013 and Ssal_phage00014 showing the putative promoter sequences of p_L and p_R. The transcription initiation sites of these two ORFs determined by 5' RACE are indicated by +1. The RBS are in italics. The extended -10 element of Ssal_phage00013 promoter (p_L) and the putative -35 and -10 elements of Ssal_phage00014 promoter (p_R) are underlined and overlined, respectively. The inverted repeats between the -10 and -35 elements, O_L and O_R, are highlighted.