A GC-Rich Prophage-Like Genomic Region of *Mycoplasma bovirhinis* HAZ141_2 Carries a Gene Cluster Encoding Resistance to Kanamycin and Neomycin.

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SUPPLEMENTAL MATERIAL

Supplementary Figure S1. **Sequence alignment of the 16S rRNA genes of *M. bovirhinis* strains and *E. coli.***

Supplementary Figure S2. **A semi-quantitative transcription analysis of *M. bovirhinis* HAZ141_2 *aadE**, *sat4* and *aphA-3* genes.**

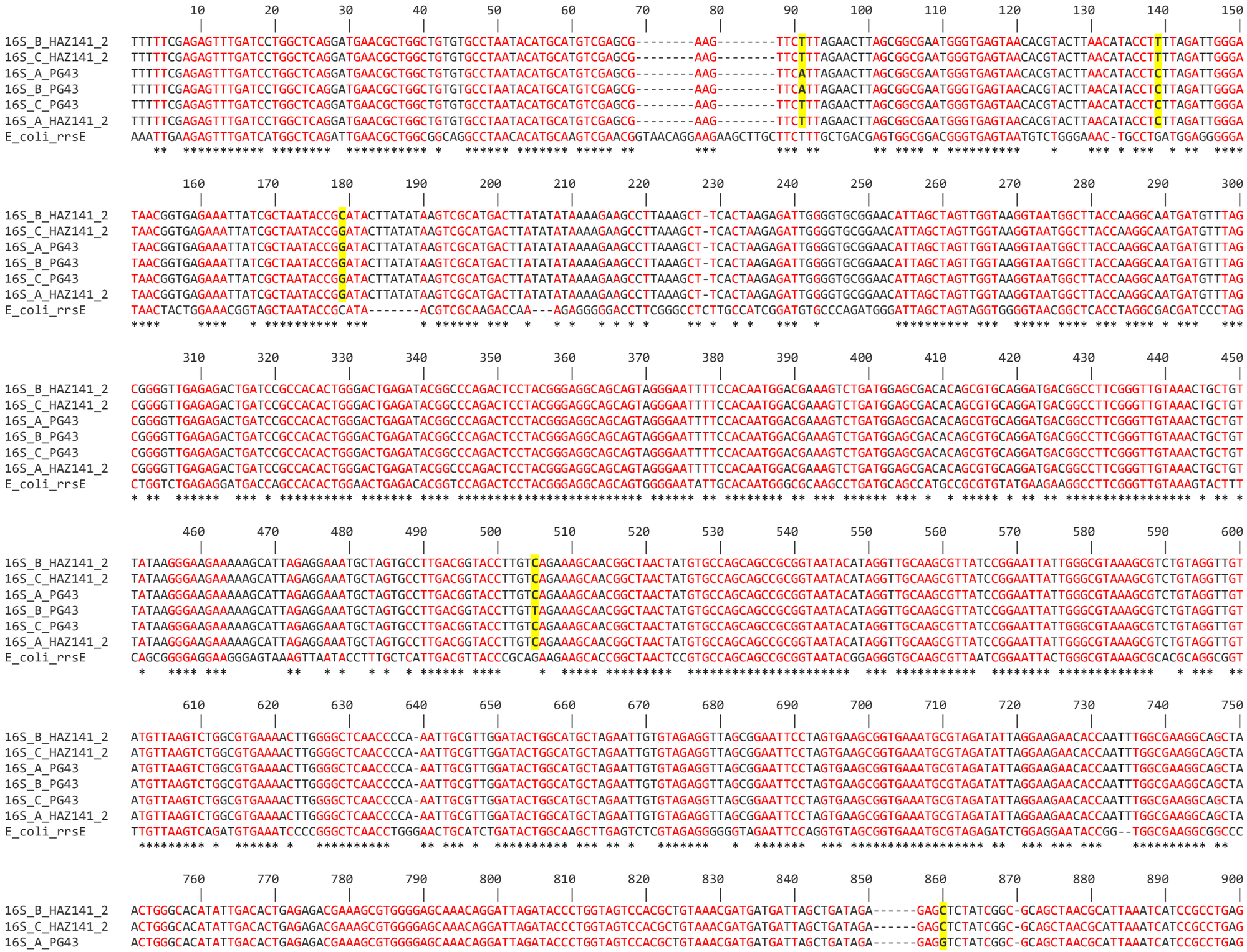
Supplementary Figure S3. **Multiple promoters can regulate expression of the *aphA-3* gene: an overview**.

Supplementary Figure S4. **Identification of the primary transcriptional start site of the *Mycoplasma bovirhinis* HAZ141_2 *aadE*-sat4-aphA-3* gene cluster (operon).**

TABLE S1. Annotation of the 53,923 bp prophage-like genomic region of *M. bovirhinis* strain HAZ141_2 (an Excel file).

TABLE S2. Oligonucleotides used in this study.

Figure S1


16S_B_HAZ141_2
16S_C_HAZ141_2
16S_A_PG43
16S_B_PG43
16S_C_PG43
16S_A_HAZ141_2
E_coli_rrsE

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16S_B_PG43      ACTGGGCACATATTGACACTGAGAGACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGATGATTAGCTGATAGA------GAGGTCTATCGGC-GCAGCTAACGCATTAAATCATCCGCCTGAG
16S_C_PG43      ACTGGGCACATATTGACACTGAGAGACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGATGATTAGCTGATAGA------GAGGTCTATCGGC-GCAGCTAACGCATTAAATCATCCGCCTGAG
16S_A_HAZ141_2  ACTGGGCACATATTGACACTGAGAGACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGATGATTAGCTGATAGA------GAGGTCTATCGGC-GCAGCTAACGCATTAAATCATCCGCCTGAG
E_coli_rrsE     CCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGG
                 **** *  * * **** ** **   ********************************************** **********  ** * *  * * *       ***   *  *  * * ********* **** **  ******* *

                       910       920       930       940       950       960       970       980       990      1000      1010      1020      1030      1040      1050
                        |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
16S_B_HAZ141_2  TAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGATCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGTAGAACCTTACCCACTCTTGACATCTTCTGCAAAGCTATAGAGATATAGCAGAGGTT-----AA
16S_C_HAZ141_2  TAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGATCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGTAGAACCTTACCCACTCTTGACATCTTCTGCAAAGCTATAGAGATATAGCAGAGGTT-----AA
16S_A_PG43      TAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGATCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGTAGAACCTTACCCACTCTTGACATCTTCTGCAAAGCTATAGAGATATAGCAGAGGTT-----AA
16S_B_PG43      TAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGATCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGTAGAACCTTACCCACTCTTGACATCTTCTGCAAAGCTATAGAGATATAGCAGAGGTT-----AA
16S_C_PG43      TAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGATCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGTAGAACCTTACCCACTCTTGACATCTTCTGCAAAGCTATAGAGATATAGCAGAGGTT-----AA
16S_A_HAZ141_2  TAGTATGCTCGCAAGAGTGAAACTTAAAGGAATTGACGGGGATCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGAAGATACGCGTAGAACCTTACCCGCTCTTGACATCTTCTGCAAAGCTATAGAGATATAGCAGAGGTT-----AA
E_coli_rrsE     GAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGG-AAGTTTTCAGAGATGAGAATGTGCCTTCGGGAA
                 **** *  ******  * ***** *** ************  ******************************** ** *  ***** ***********   **********  * * **   *  ******     * *  *    **

                      1060      1070      1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
                        |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
16S_B_HAZ141_2  CAGAATGACAGATGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTCGGTTAAGTCCTGCAACGAGCGCAACCCTTGTCCTTAGTTAATT---------------TTTAGGGAGACTGCCCGAG-TAATTGGGAGGAAGGTGGG
16S_C_HAZ141_2  CAGAATGACAGATGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTCGGTTAAGTCCTGCAACGAGCGCAACCCTTGTCCTTAGTTAATT---------------TTTAGGGAGACTGCCCGAG-TAATTGGGAGGAAGGTGGG
16S_A_PG43      CAGAATGACAGATGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTCGGTTAAGTCCTGCAACGAGCGCAACCCTTGTCCTTAGTTAATT---------------TTTAGGGAGACTGCCCGAG-TAATTGGGAGGAAGGTGGG
16S_B_PG43      CAGAATGACAGATGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTCGGTTAAGTCCTGCAACGAGCGCAACCCTTGTCCTTAGTTAATT---------------TTTAGGGAGACTGCCCGAG-TAATTGGGAGGAAGGTGGG
16S_C_PG43      CAGAATGACAGATGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTCGGTTAAGTCCTGCAACGAGCGCAACCCTTGTCCTTAGTTAATT---------------TTTAGGGAGACTGCCCGAG-TAATTGGGAGGAAGGTGGG
16S_A_HAZ141_2  CAGAATGACAGATGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTCGGTTAAGTCCTGCAACGAGCGCAACCCTTGTCCTTAGTTAATT---------------TTTAGGGAGACTGCCCGAG-TAATTGGGAGGAAGGTGGG
E_coli_rrsE     CCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGG
                 * *   ***** ** ******* ***************** **** ***** ********** ****************** ***** ***                    *  * ********** * * ***   **************

                      1210      1220      1230      1240      1250      1260      1270      1280      1290      1300      1310      1320      1330      1340      1350
                        |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
16S_B_HAZ141_2  GACGACGTCAAATCATCATGCCTCTTACGAGTGGGGCAACACACGTGCTACAATGGATAGTACAAAGAGAAGCAACATGGTGACATCAAGCAAATCTCAAAAA-CTATTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGTC
16S_C_HAZ141_2  GACGACGTCAAATCATCATGCCTCTTACGAGTGGGGCAACACACGTGCTACAATGGATAGTACAAAGAGAAGCAACATGGTGACATCAAGCAAATCTCAAAAAACTATTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGTC
16S_A_PG43      GACGACGTCAAATCATCATGCCTCTTACGAGTGGGGCAACACACGTGCTACAATGGATAGTACAAAGAGAAGCAACATGGTGACATCAAGCAAATCTCAAAAA-CTATTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGTC
16S_B_PG43      GACGACGTCAAATCATCATGCCTCTTACGAGTGGGGCAACACACGTGCTACAATGGATAGTACAAAGAGAAGCAACATGGTGACATCAAGCAAATCTCAAAAA-CTATTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGTC
16S_C_PG43      GACGACGTCAAATCATCATGCCTCTTACGAGTGGGGCAACACACGTGCTACAATGGATAGTACAAAGAGAAGCAACATGGTGACATCAAGCAAATCTCAAAAA-CTATTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGTC
16S_A_HAZ141_2  GACGACGTCAAATCATCATGCCTCTTACGAGTGGGGCAACACACGTGCTACAATGGATAGTACAAAGAGAAGCAACATGGTGACATCAAGCAAATCTCAAAAA-CTATTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGTC
E_coli_rrsE     GATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTC
                 ** ******** ******** * *******   **** ******************    ************* ** * * ** * *****  * **** ***     **  *** ******* ********** *********

                      1360      1370      1380      1390      1400      1410      1420      1430      1440      1450      1460      1470      1480      1490      1500
                        |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
16S_B_HAZ141_2  GGAATCGCTAGTAATCGTAGATCAGCTACGCTACGGTGAATACGTTCTCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCTGGTAATGCCCGAAGTCGGTTTTGTTAACTACGG-AGACAACTGCCTAAGGCAGGGCCGGTGACT
16S_C_HAZ141_2  GGAATCGCTAGTAATCGTAGATCAGCTACGCTACGGTGAATACGTTCTCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCTGGTAATGCCCGAAGTCGGTTTTGTTAACTACGG-AGACAACTGCCTAAGGCAGGGCCGGTGACT
16S_A_PG43      GGAATCGCTAGTAATCGTAGATCAGCTACGCTACGGTGAATACGTTCTCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCTGGTAATGCCCGAAGTCGGTTTTGTTAACTACGG-AGACAACTGCCTAAGGCAGGGCCGGTGACT
16S_B_PG43      GGAATCGCTAGTAATCGTAGATCAGCTACGCTACGGTGAATACGTTCTCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCTGGTAATGCCCGAAGTCGGTTTTGTTAACTACGG-AGACAACTGCCTAAGGCAGGGCCGGTGACT
16S_C_PG43      GGAATCGCTAGTAATCGTAGATCAGCTACGCTACGGTGAATACGTTCTCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCTGGTAATGCCCGAAGTCGGTTTTGTTAACTACGG-AGACAACTGCCTAAGGCAGGGCCGGTGACT
16S_A_HAZ141_2  GGAATCGCTAGTAATCGTAGATCAGCTACGCTACGGTGAATACGTTCTCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCTGGTAATGCCCGAAGTCGGTTTTGTTAACTACGG-AGACAACTGCCTAAGGCAGGGCCGGTGACT
E_coli_rrsE     GGAATCGCTAGTAATCGTGGATCAGA-ATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACT
                 ****************** ******  * ** ************** **** ********************************  ***       ***** ***    * * ** *** **    **  * *      *     *****

                      1510      1520      1530      1540      1550
                        |         |         |         |         |
16S_B_HAZ141_2  GGGGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATTACCTCCTTT
16S_C_HAZ141_2  GGGGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATTACCTCCTTT
16S_A_PG43      GGGGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATTACCTCCTTT
16S_B_PG43      GGGGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATTACCTCCTTT
16S_C_PG43      GGGGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATTACCTCCTTT
16S_A_HAZ141_2  GGGGTGAAGTCGTAACAAGGTATCCCTACGAGAACGTGGGGATGGATTACCTCCTTT
E_coli_rrsE     GGGGTGAAGTCGTAACAAGGTAACCGTAGGGGAACCTGCGGTTGGATCACCTCCTTA
                ********************** ** ** * **** ** ** ***** ********
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Figure S1. Sequence alignment of the 16S rRNA genes of *M. bovirhinis* strains and *E. coli.* Sequences of three alleles of the16S rRNA genes were extracted from the genomes of *M. bovirhinis* PG43 type strain (NCTC 10118/ATCC 27748; accession no. LR214972.1) as well as the Japanese *M. bovirhinis* isolate HAZ141_2 (AP018135.1). The *rrsE* gene of *E. coli* K-12 strain MG1655 (NC_000913.2) was used as a reference. The multiple alignments was conducted by CLUSTAL W (Thompson et al., 1994) using on-line Clustal server hosted at https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalwan.html. Alignment length is 1557 nucleotides including 1107 (71.10%) identical nucleotides shown in red and marked with asterisk (*), while 450 (28.90%) different nucleotides are shown in black. Nucleotides differed between *M. bovirhinis* PG43 and HAZ141_2 strains are bolded and yellow-highlighted, while the nucleotides affect sensitivity to aminoglycosides are shown in bold and blue-highlighted.

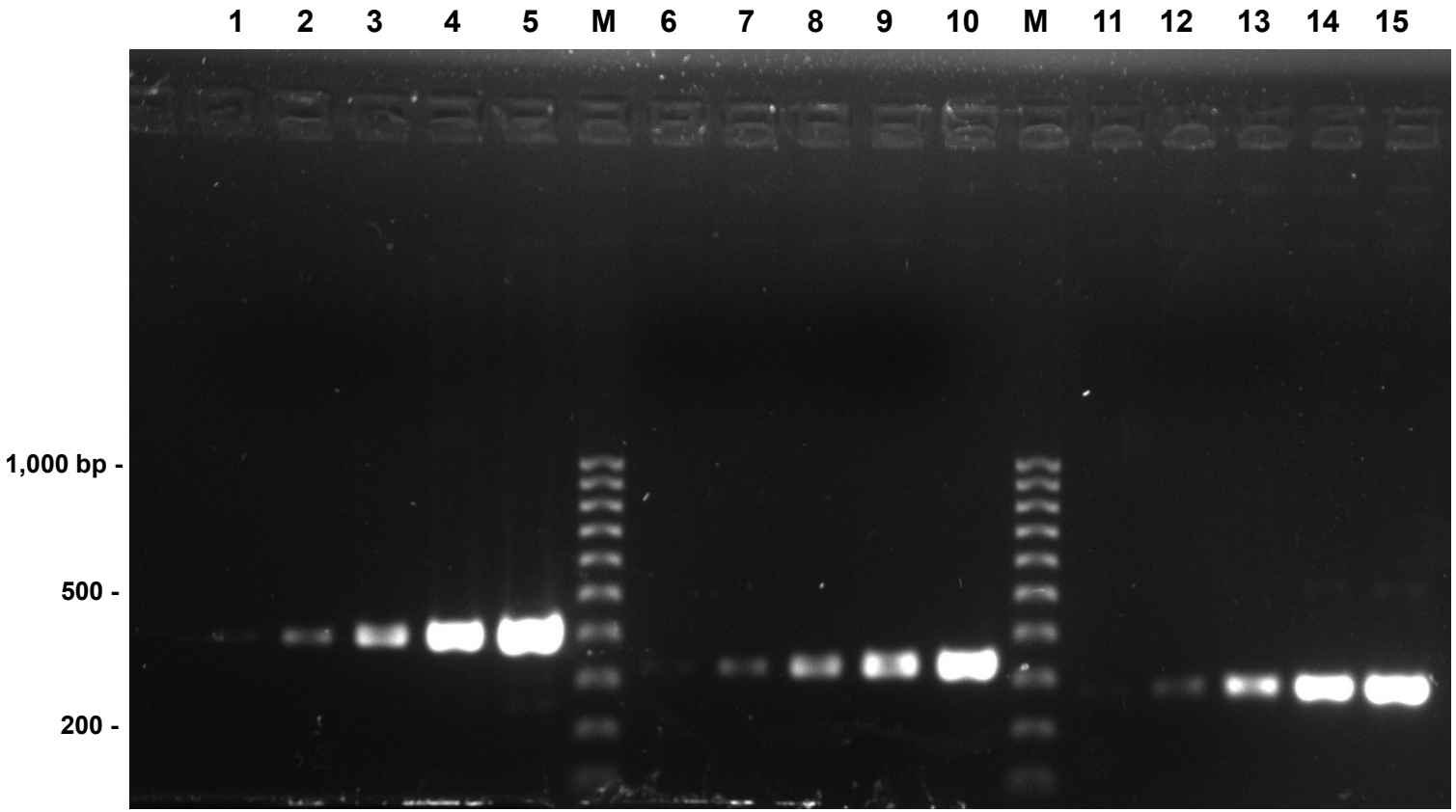
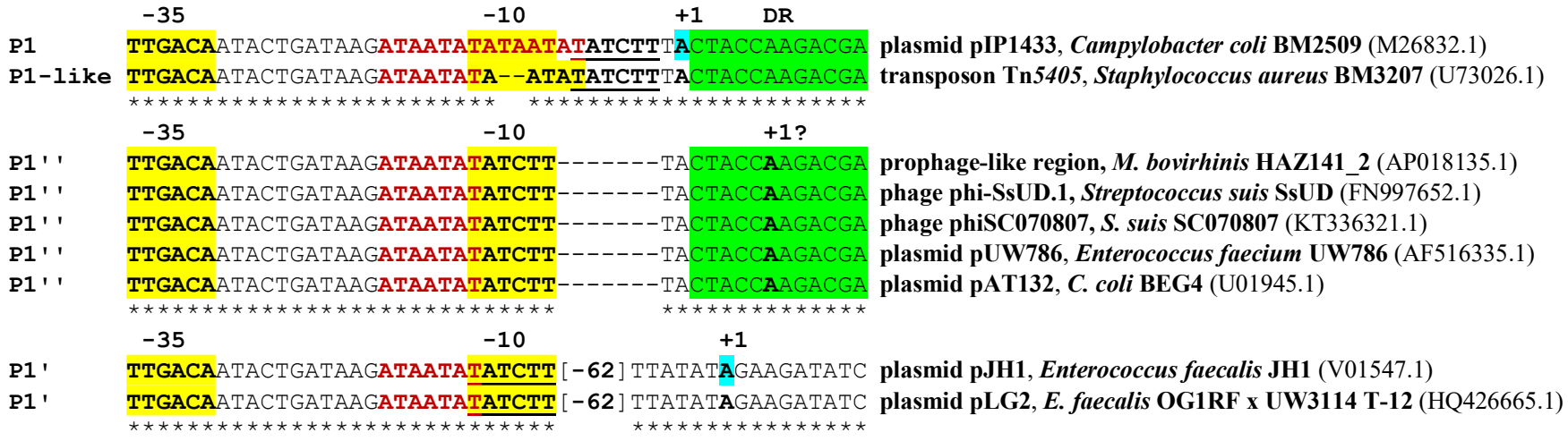


Figure S2. A semi-quantitative transcription analysis of *M. bovirhinis* HAZ141_2 *aadE, *sat4* and *aphA-3* genes.** Agarose gel electrophoresis of RT-PCR products. cDNAs, synthesized with either aadE-R1, or sat4-R1, or aphA3-R2 primers complementary to the *aadE*, sat4* and *aphA-3* genes, respectively, were subjected to PCR amplification using different number of cycles to determine a pattern of expression of each individual gene. **1-5**: PCR with primers aadE-F2 and aadE-R1 performed on cDNA obtained with aadE-R1. **6-10**: PCR with primers sat4-F1 and sat4-R1 performed on cDNA obtained with sat4-R1. **11-15**: PCR with primers aphA3-F1 and aphA3-R2 performed on cDNA obtained with aphA3-R2. The numbers of PCR cycles were as a follow: 22 – lanes 1, 6 and 11; 24 – lanes 2, 7 and 12; 26 – lanes 3, 8 and 13; 28 – lanes 4, 9 and 14; 30 – lanes 5, 10 and 15. The 100-bp ladder (BioRad, California, USA) is shown as M.

Figure S3

'Internal' homologous promoters of *aphA-3* located within a *sat4* ORF.



Promoter P2 located within an intergenic region - between *sat4* and *aphA-3*.

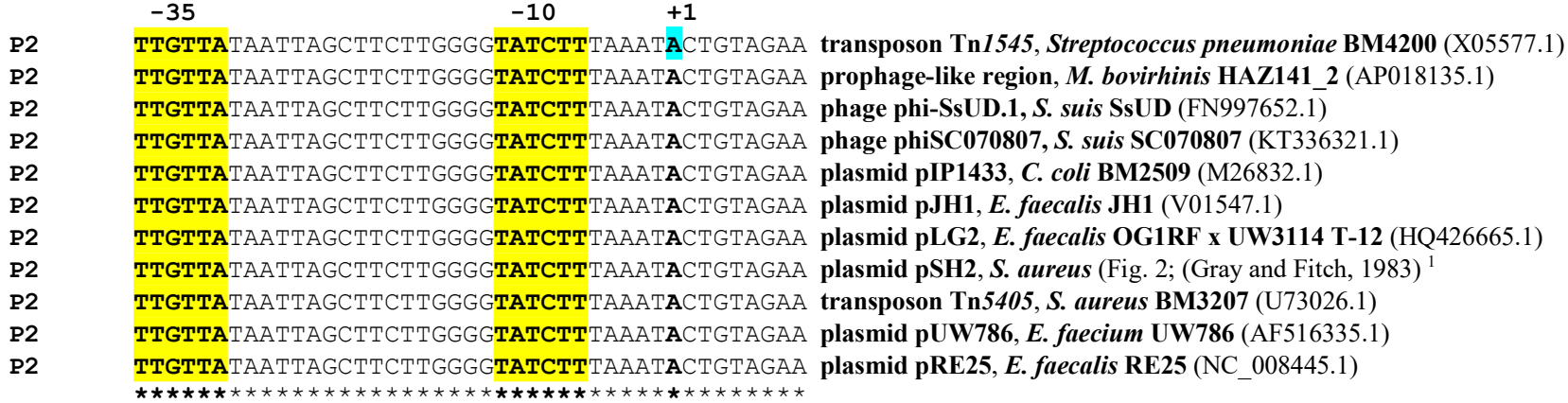
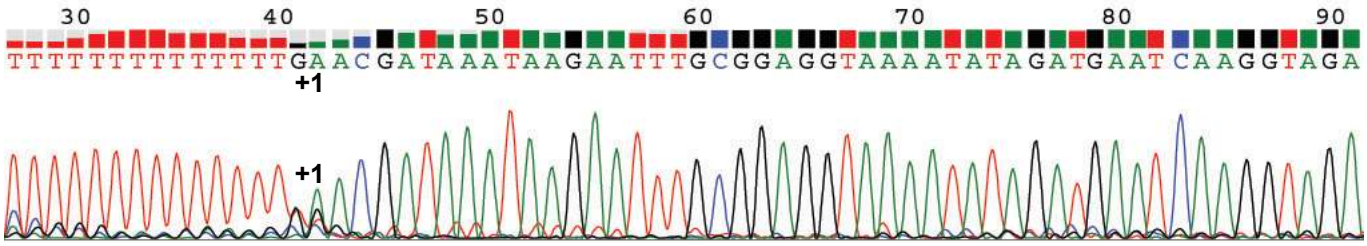


Figure S3. **Multiple promoters can regulate expression of the *aphA-3* gene: an overview**. Sequence features of *aphA-3* promoters are shown as following: both -35 and -10 elements are yellow high-lighted; 7 bp sequence ATAATAT is shown in red bold (twice in P1 promoter of plasmid pIP1433, an upper line); experimentally detected transcriptional start sites (+1) are shown in bold and blue high-lighted (Caillaud et al., 1987; Trieu-Cuot et al., 1985; Gray and Fitch, 1983); P1'' predicted transcriptional start site is shown in bold and marked as +1?; one of two 12 bp direct repeats (DRs) flanking both P1 and P1'' promoters is shown in bold and green high-lighted. Both -10 element of P1' promoter and its orthologous sequence of P1 are additionally underlined. A particular 62 bp deletion within *sat4* ORF of some bacteria is shown as -62 in square brackets. Promoter P1'' is found only in within an intact *sat4* ORF. DNA sequence compilation based on previously reported data (Caillaud et al., 1987; Trieu-Cuot et al., 1985; Gray and Fitch, 1983), and retrieved from GenBank (corresponding accession numbers are given in brackets). [1] The DNA sequence of plasmid pSH2 is reproduced as it shown in Fig.2 (Gray and Fitch, 1983).

A



B

-35 ext. -10 +1 *RBS* *aadE** →

TTGAAAAAATGTAAAATTTG**TGATATAAT**AAACT**G**AACGATAAATAAGAATTTGC*GGAGGT*AAAATATAG*ATG*AATCAAGGTAGA...

M N Q G R

C

	-35	14 nts	ext. -10		+1		fMet	
aadE*	**TTGAAA**	AAATGTAAAATTTG	**TGATATAAT**	AAACT	**GA**	34 bp	***ATG***	228 aa ORF (**1**)
aadE*	**TTttAA**	AAtaGTAgAcTaTG	**TGATATAAT**	AgAaTc	**G**	23 bp	***ATG***	206 aa ORF (**2**)
aadE*	**TTttAA**	AAtaGTAgAcTaTG	**TGATATAAT**	AgAaTc	**G**	23 bp	***ATG***	206 aa ORF (**3**)
aadE*	**TTGAAA**	AGtgGatAgATTTa	**TGATAcAgT**	gAAta	**GA**	53 bp	***ATa***	206 aa ORF (**4**)
	-35	**15 nts**	**ext. -10**		**+1?**		**fMet**	
aadE	**TTaAaA**	AAATcgttttaTgGt	**TGtTcaAAT**	tAtCCT	**GA**	48 bp	***ATG***	302 aa ORF (**5**)
aadE1	**TTaAaA**	AAATcgttttaTgGt	**TGtTcaAAT**	tAtCCT	**GA**	48 bp	***ATG***	287 aa ORF (**6**)
aadE2	**TTGtat**	cggcaaAAAAgaaGa	**TGtaATAAT**	AtAgAA	**A**	21 bp	***ATG***	302 aa ORF (**6**)
aadE	**TTGtat**	cggcaaAAAAgaaGa	**TGtaATAAT**	AtAgAA	**A**	21 bp	***ATG***	302 aa ORF (**7**)
aadE	**TTGtat**	cggcaaAAAAgaaGa	**TGtaATAAT**	AtAgAA	**A**	21 bp	***ATG***	302 aa ORF (**8**)

Figure S4. **Identification of the primary transcriptional start site of the *Mycoplasma bovirhinis* HAZ141_2 *aadE*-sat4-aphA-3* gene cluster (operon). (A)** A partial sequence chromatogram of a (+) strand of the final 5'-RACE PCR product amplified with primers Anchor (Roche Diagnostics GmbH, Mannheim, Germany) and aadE-R3 (see for details Materials and Methods). The determined alternative transcriptional start sites (+1) are represented by two nucleotides (nts) - G and A. **(B) A deduced RpoD-dependent promoter of the *M. bovirhinis* HAZ141_2 *aadE*-sat4-aphA-3* gene cluster (operon)**. The -35 and extended -10 (ex. -10) elements of the RpoD-dependent promoter are shown in bold and yellow high-lighted; its cognate 2-nt transcriptional start site (as +1 and underlined) is located 35-36 nts upstream of the first transcriptional codon (ATG) that shown in italic and underlined, while the predicted ribosome-binding site (RBS) is shown in italic. Nts including +1 and DNA sequence downstream of it are shown in bold and colorized according to panel A. MNQGR sequence is presenting N-terminal amino acid residues of AadE-like protein encoded by *aadE**. (**C**) Conservation of *aadE*-like gene promoter regions in different bacterial replicons and mobile elements. Numbers shown in brackets after ORF are the following: 1 - prophage-like, *M. bovirhinis* HAZ141_2 (AP018135.1); 2 - chromosome, *Streptococcus pyogenes* NGAS322 (CP010449.1); 3 - chromosome, *S. pyogenes* GA40056 (AWTI01000014.1); 4 - plasmid pCG8245, *Campylobacter jejuni* CG8245 (AY701528.1); 5 - phage phi-SsUD.1, *Streptococcus suis* SsUD (FN997652.1); 6 - plasmid pXD5, *Enterococcus faecium* P26 (KJ645709.1); 7 - transposon *Tn5405*, *Staphylococcus aureus* BM3207 (the DNA sequence derived from Fig. 2, Derbise et al., 1997); 8 - plasmid pUW786, *E. faecium* UW786 (AF516335.1). Capital letters used for identical nts, while lower-case letters for non-conserved nts.

TABLE S2 Oligonucleotides used in this study

Designation	Sequence (5' ➔ 3') [a]	Purpose of use
aadE-F1-SphI	attaGCATGCGCACATTCGGTAACGGAAGC	Amplification of *aadE*-sat4-aphA-3* genes for cloning into the pACYC184 derivative
aphA3-R1-HindIII	attaAAGCTTTTTAGATATCTAAATCTAGG	
pACYC184-F1-HindIII	ACTACCGCATTAAAGCTTATCG	Amplification of a pACYC184 derivative (without *tetA* gene) and sequence verification of the recombinant plasmid pAC10
pACYC184-R1-SphI	tataGCATGCGTATTAACGAAGCGCTAACC	
pACYC184-ori-R1	CAAGAGATTACGCGCAGACC	Sequence verification of the recombinant plasmid pAC10
pACYC184-F1	AACGCCTGGTGCTACGCCTG	
pACYC184-cat-R1	TGAGCATTCATCAGGCGGGC	
pACYC184-ori-R2	GGAACTGAGTGTCAGGCGTGG	
aadE-F2	AACGGCATCTGCTGTTGC	PCRs on cDNA obtained with primer aadE-R1 to determine expression as well as relative expression of *aadE** mRNA
aadE-F3	ATGGAAGGTCGGCATCGA	PCR on cDNA obtained with aphA3-R2 primer to determine co-expression of *aadE*-sat4-aphA-3*
aadE-R1	CGGATACCGCCCTGAACAAT	Sequence verification of the recombinant plasmid pAC10; cDNA amplification of *aadE** mRNA either for RT-PCR or 5'-RACE; PCR on cDNA obtained with primer aadE-R1 to determine relative expression of *aadE** mRNA
aadE-R2	AAAGCCTGTTTCGATGCCGACC	PCR on cDNA obtained with primer aadE-R1 to determine expression of *aadE** mRNA; first 5'-RACE PCR
aadE-R3	ATCATCATACTCCCTTGCGC	Nested 5'-RACE PCRs; sequencing of 5'-RACE products
sat4-F1	AAGACGAAGAGGATGAAGAGG	PCRs on cDNA obtained with sat4-R1 primer to determine expression as well as relative expression of *sat4* mRNA
sat4-R1	TAACATAGTATCGACGGAGCCG	cDNA amplification of *sat4* mRNA either for RT-PCR or 5'-RACE; PCRs on cDNA obtained with sat4-R1

		primer to determine expression as well as relative expression of *sat4* mRNA
sat4-R2	TATAAGCGTACCGGTTCC	PCR on cDNA obtained with aphA3-R2 primer to determine co-expression of *aadE*-sat4* mRNA
sat4-R3	TTGTCCTGGGTTTCAAGC	First 5'-RACE PCR
aphA3-F1	GGACATGATGCTATGGCTGG	Sequence verification of the recombinant plasmid pAC10; PCRs on cDNA obtained with aphA3-R2 primer to determine expression as well as relative expression of *aphA-3* mRNA
aphA3-F2	GGGGATCAAGCCTGATTGGG	Sequence verification of the recombinant plasmid pAC10
aphA3-R1	CCAGCCATAGCATCATGTCC	PCR on cDNA obtained with aphA3-R2 primer to determine co-expression of *aadE*-sat4-aphA-3* mRNA; first 5'-RACE PCR
aphA3-R2	ATCCACATCGGCCAGATCG	cDNA amplification of *aphA-3* mRNA either for RT-PCR or 5'-RACE; PCR on cDNA obtained with primer aphA3-R2 to determine expression as well as relative expression of *aphA3* mRNA

[a] *Hind*III and *Sph*I sites are underlined, while 5' nucleotides (added to improve restriction cleavage) are shown as lowercase letters.

REFERENCES (for Supplemental material)

Caillaud F, Trieu-Cuot P, Carlier C, Courvalin P. 1987. Nucleotide sequence of the kanamycin resistance determinant of the pneumococcal transposon Tn*1545*: evolutionary relationships and transcriptional analysis of *aphA-3* genes. Mol Gen Genet 207:509-513.
(**Fig. 1**: **P1** and **+1; *aphA-3* gene**; plasmid pIP1433, *Campylobacter coli* BM2509; **P2** and **+1; *aphA-3* gene**; transposon Tn*1545*, *Streptococcus pneumoniae* BM4200).

Chen S, Hao H, Zhao P, Liu Y, Chu Y. 2018. Genome-wide analysis of *Mycoplasma bovirhinis* GS01 reveals potential virulence factors and phylogenetic relationships. G3 (Bethesda) 8:1417-1424.
(**Table S1**).

Derbise A, de Cespedes G, el Solh N. 1997. Nucleotide sequence of the *Staphylococcus aureus* transposon, Tn*5405*, carrying aminoglycosides resistance genes. J Basic Microbiol 37:379-384.

Gray GS, Fitch WM. 1983. Evolution of antibiotic resistance genes: the DNA sequence of a kanamycin resistance gene from *Staphylococcus aureus*. Mol Biol Evol 1:57-66.
(**Fig. 2**: **P2 promoter region of *aphA-3***, plasmid pSH2, *Staphylococcus aureus*).

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673-4680.

Trieu-Cuot P, Klier A, Courvalin P. 1985. DNA sequences specifying the transcription of the streptococcal kanamycin resistance gene in *Escherichia coli* and *Bacillus subtilis*. Mol Gen Genet 198**:**348-352.
(**P1'** and **+1; *aphA-3* gene**; plasmid pJH1, *Enterococcus faecalis* JH1).