Supplementary material

Antimicrobial Peptide Functionalized Polyhydroxyalkanoate Bio-beads as A Bactericidal Material for Water Disinfection

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Table S1. Plasmids and primers used in this study.

Plasmids and primers	Description	Reference
Plasmids		
pET23b(+)	Bacterial vector for protein expression	1
pBBR1MCS-2	Bacterial vector for cloning and protein expression	2
pET23b-HHC10-phaC	*HHC10-phaC* expressed in pET23b(+)	This study
pET23b-HA-HHC10-phaC	HA tagged *HHC10-phaC* expressed in pET23b(+)	This study
pBBR-phaAB	*phaA* and *phaB* expressed in pBBR1MCS-2-kan	This study
Primers		
NdeI_HHC10_G4S_phaC_F	gggcatatgaaacgctggtggaaatggattcgctgggggggggggaggaagtgc gaccggcaaaggc	
NdeI-HA-HHC10_PhaC_F	gggcatatgtacccatacgacgttccagactacgctaaacgctggtggaaatgga ttcgctggctggtgccgcgcggcagcgcgaccggcaaaggc	
phaC_BamHI_R	cccggatcctcatgccttggctttgacg	
phaAB_XhoI_F	gggctcgagaaggagatataccatgactgacgttgtcatcgtatc	
phaAB_BamHI_R	cccggatcctcagcccatatgcaggc	

Table S2. Characteristics of the surface water samples from South Bend, Indiana.

	Surface Water	
Parameters (mg/L)	**SM**	**SJ**
Ionic strength (M)	0.010	0.013
pH	8.75	8.60
Na^{+}	143.65	192.58
Ca^{2+}	33.2	37.05
Mg^{+}	21.56	26.23
K^{+}	4.78	5
Cu^{2+}	0.0067	0.049
Fe^{3+}	ND	0.0057
Zn^{2+}	ND	0.00068
F^{-}	0.11	0.11
Cl^{-}	180.53	221.36
NO_2^{-}	0.59	ND
Br^{-}	2.21	2.2
NO_3^{-}	1.27	1.63
PO_4^{3-}	ND	ND
SO_4^{2-}	44.61	60.09

Abbreviations: ND, not detected; SM, St. Mary's Lake; SJ, St. Joseph's Lake.

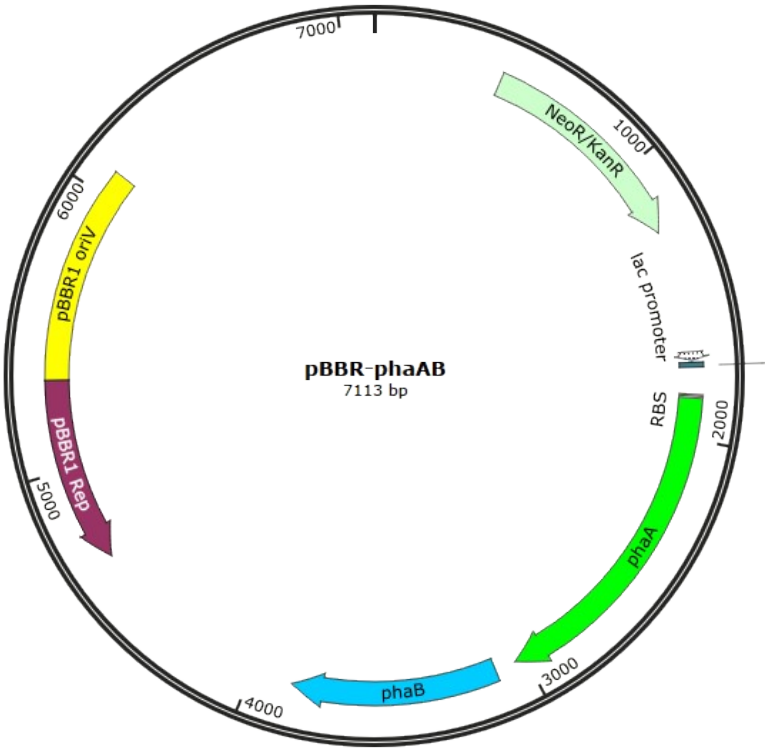


Figure S1. The plasmid map of pBBR-phaAB.

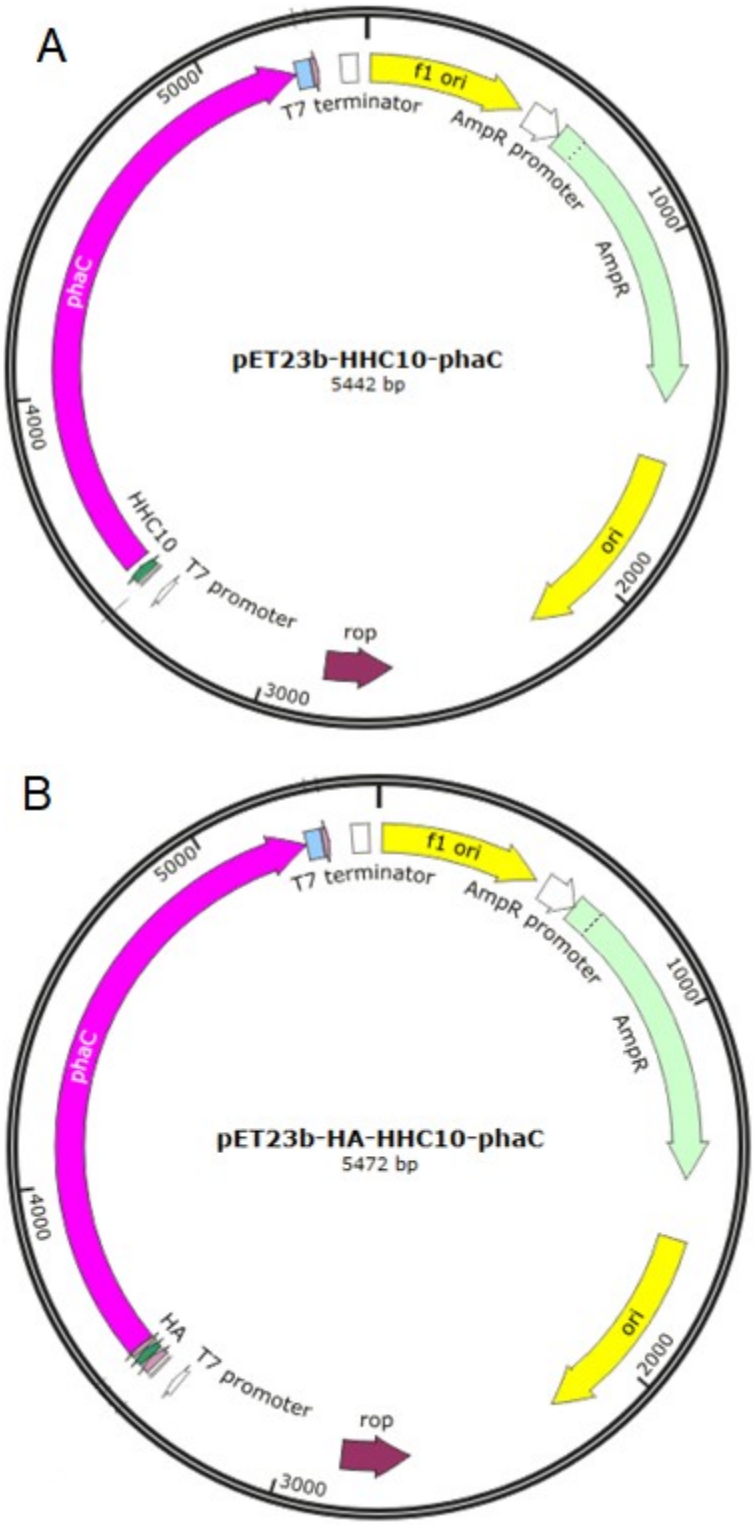
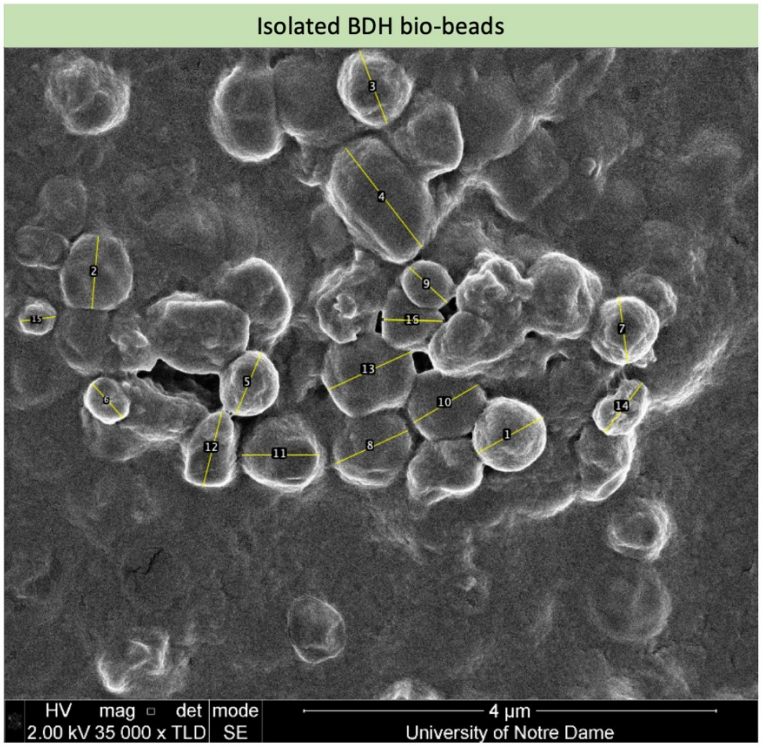


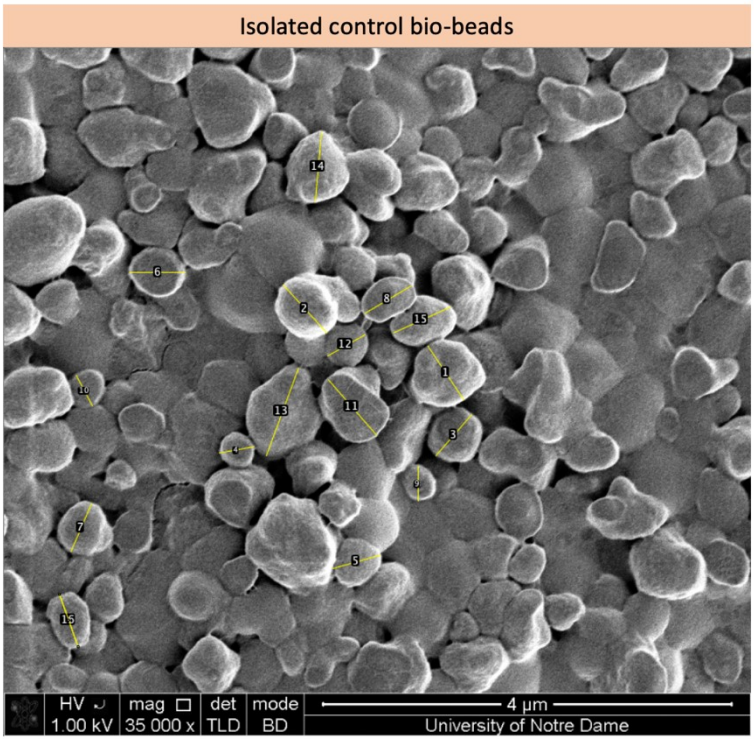
Figure S2. The plasmid map of p23b-HHC10-phaC (A) and p23b-HA-HHC10-phaC (B).

A



BDH number	Length (nm)
1	700
2	698
3	742
4	1206
5	630
6	454
7	633
8	765
9	507
10	719
11	745
12	744
13	880
14	596
15	333
16	592

B



Control bio-beads Number	Length (nm)
1	621
2	629
3	529
4	339
5	474
6	547
7	503
8	529
9	341
10	331
11	715
12	427
13	908
14	671
15	611
16	535

Figure S3. Approximated size of isolated BDH (A) and control bio-beads (B) in the SEM images analyzed by Fiji image software.

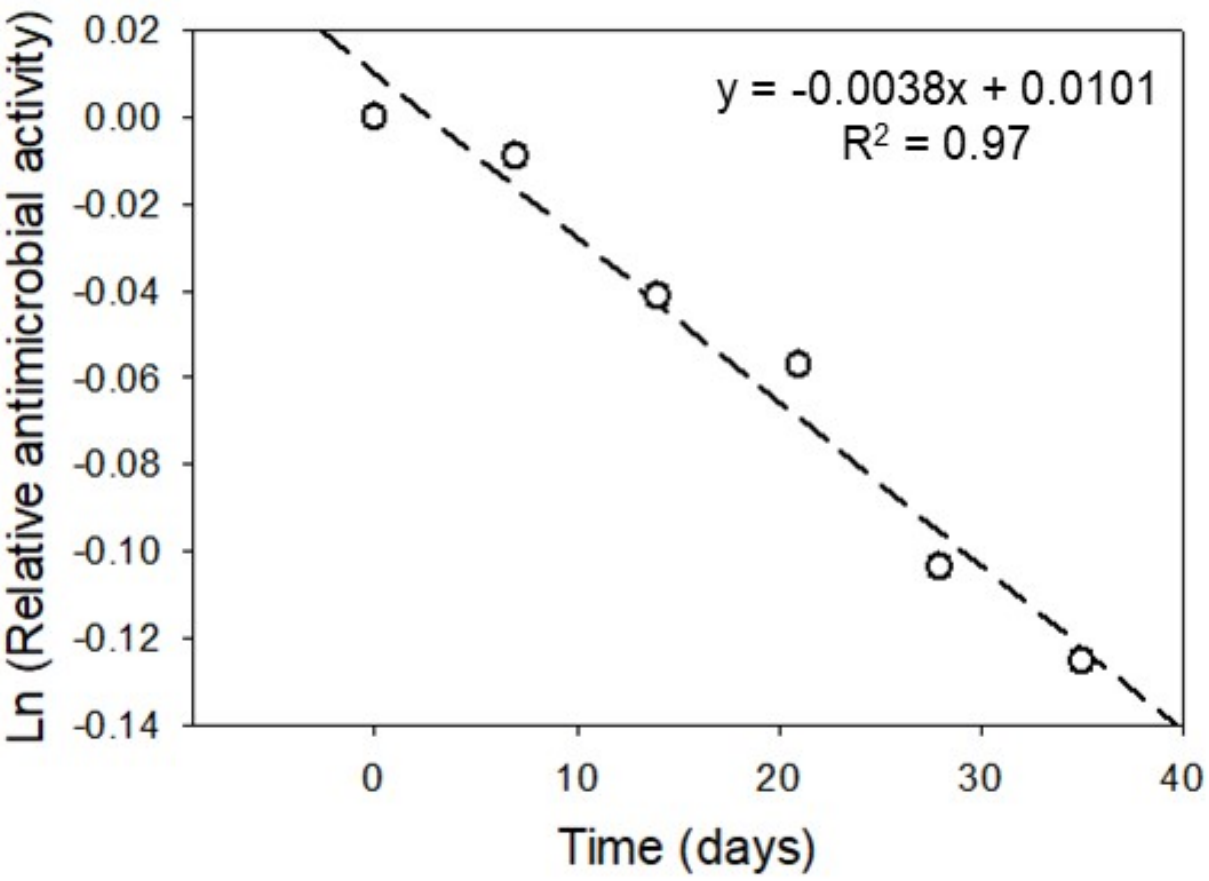


Figure S4. The natural logarithm of BDH antimicrobial activity versus time with the fitted regression line. It demonstrates the decay of BDH antimicrobial activity fits the first-order reaction.

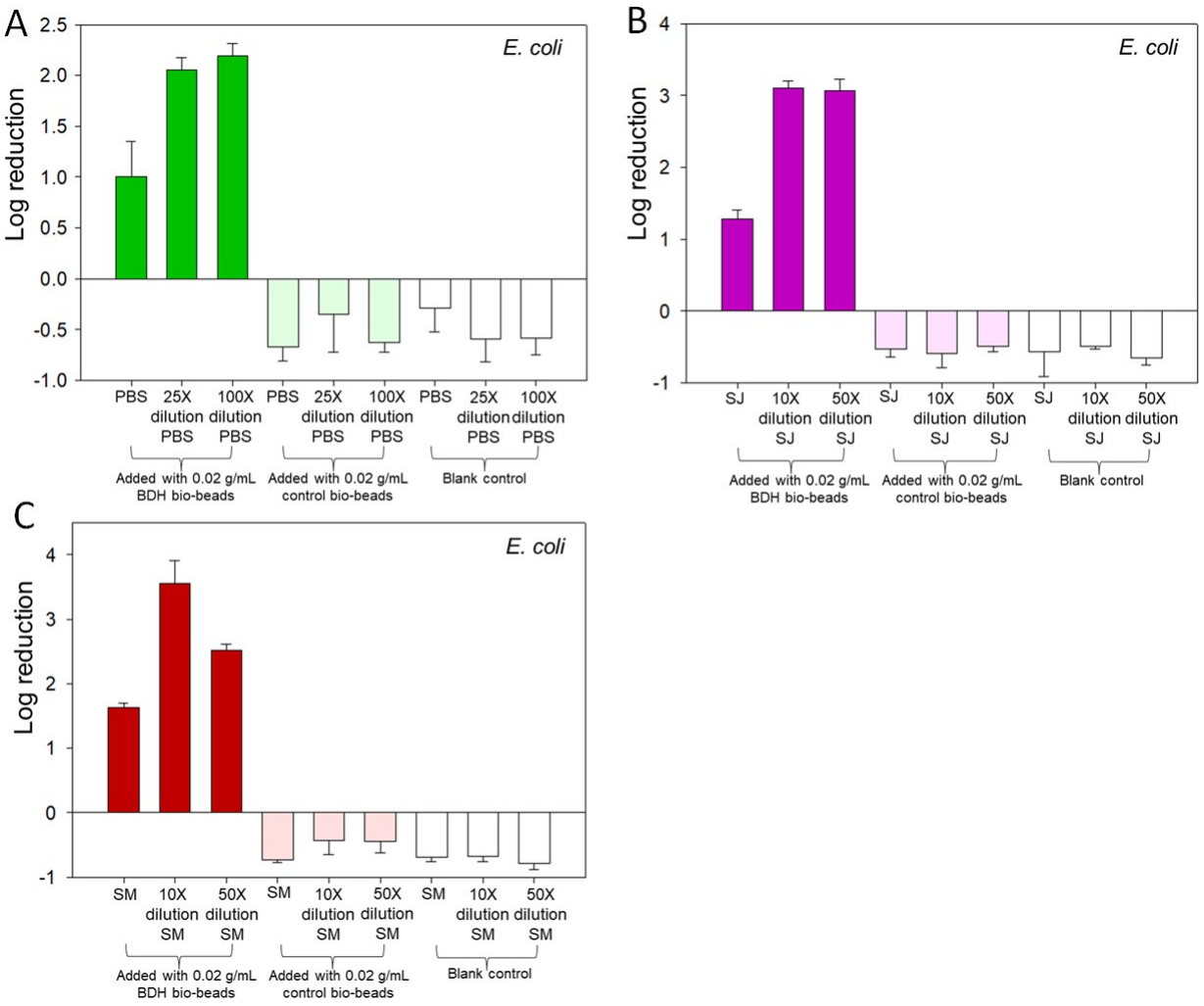


Figure S5. Log reductions of *E. coli* by the BDH, control microbeads, and blank control in PBS buffer (A), surface waters SJ (B), SM (C), and their diluted solutions. Results are the means of triplicate experiments; error bars indicating standard deviations. Abbreviations: SM, St. Mary's Lake; SJ, St. Joseph's Lake.

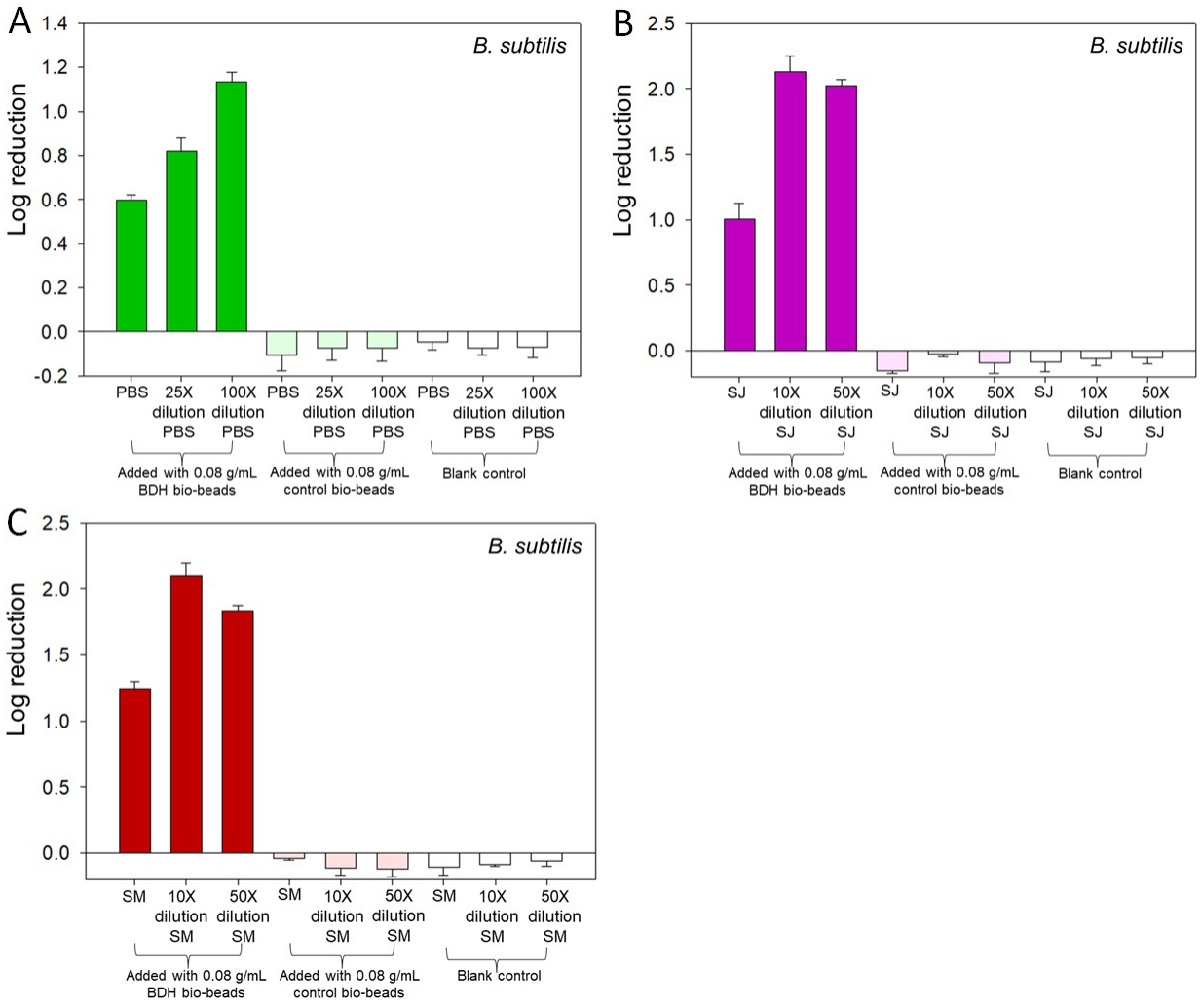


Figure S6. Log reductions of *B. subtilis* by the BDH, control microbeads, and blank control in PBS buffer (A), surface waters SJ (B), SM (C), and their diluted solutions. Results are the means of triplicate experiments; error bars indicating standard deviations. Abbreviations: SM, St. Mary's Lake; SJ, St. Joseph's Lake.

References

1. Edner, C.; Li, J.; Albrecht, T.; Mahlow, S.; Hejazi, M.; Hussain, H.; Kaplan, F.; Guy, C.; Smith, S. M.; Steup, M. Glucan, water dikinase activity stimulates breakdown of starch granules by plastidial β-amylases. *Plant Physiol.* **2007**, *145* (1), 17-28.

2. Kovach, M. E.; Elzer, P. H.; Hill, D. S.; Robertson, G. T.; Farris, M. A.; Roop II, R. M.; Peterson, K. M. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* **1995**, *166* (1), 175-176.