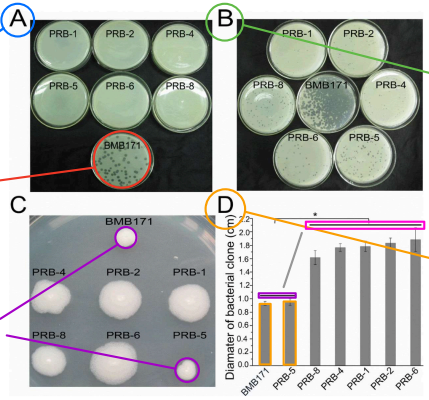


Phage-resistant bacterial mutants obtained via repeating the cultivation process o phage-containing plates more than 5 times.

Wildtype phage vB_BthS_BMBphi was only able to infect the wild type B. thuringiensis BMB171

Strains BMB171 and PRB-5 formed relatively smaller colonies on the agar plate, whereas all the other phage resistant mutants produced significantly greater clones.

Regained-infectivity phage mutants were screened via co-cultivation of the phage vB_BthS_BMBphi and phage resistant strain PRB-4 for 24 hours. All 100 picked phage isolated could infect all phage-resistnat mutants.

Statistically significant difference between clone sizes was detected between group 1 (BMB171 and PRB-5) and group 2 (PRB-8, PRB-4, PRB-1, PRB-2, and PRB-6) via SPRSS ANOVA.

Figure 1. Isolation and characterization of phage-resistant mutants of BMB171 and regained-infectivity phage mutants of vB_BthS_BMBphi. (**A**) Phage-resistant mutants of BMB171 isolated in this study. (**B**) Infectivity of regained-infectivity phage mutants vB_BthS_BMBphi-M1 to the phage-resistant strains and BMB171. (**C**) The mobility of the strains BMB171 and the phage-resistant mutants. The agar plate with a concentration of 0.3% was used for bacterial mobility analysis. (**D**) Comparison of the bacterial clone size of strain BMB171 and the phage-resistant mutants. For each strain, 30 clones were used for the analysis of the clone diameters, and the asterisk indicates a significant difference ($p < 0.05$) between the diameter of different bacterial clones.

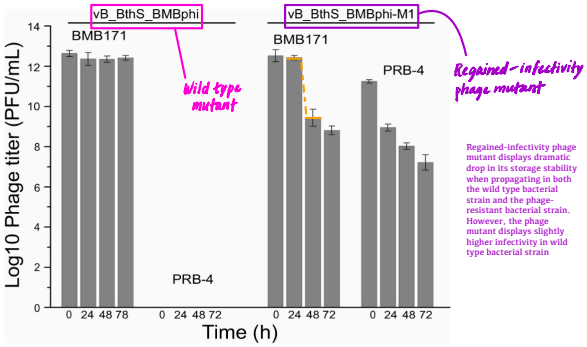


Figure 2. Storage stability of the phage vB_BthS_BMBphi and vB_BthS_BMBphi-M1. The infectivity of the two phages was tested against the strains BMB171 and PRB-4, respectively, after storage for 24, 48, and 72 h.

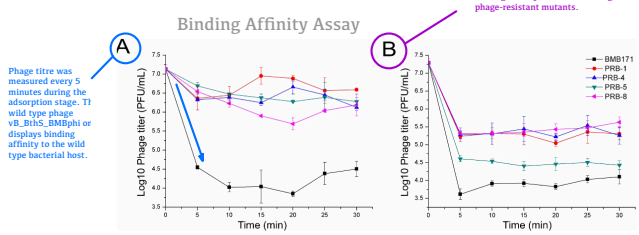


Figure 3. Absorption of phage vB_BthS_BMBphi (**A**) and vB_BthS_BMBphi-M1 (**B**) to strain BMB171 and four phage-resistant mutants.

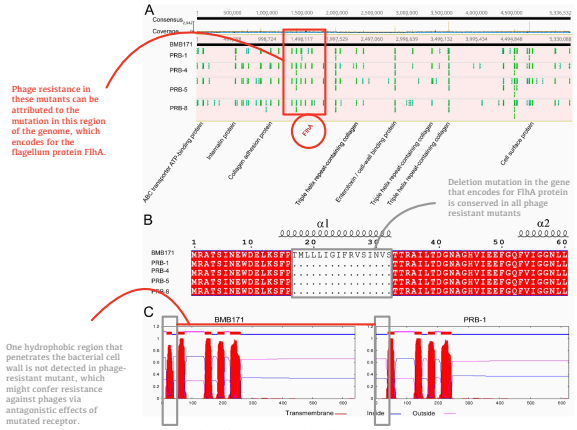


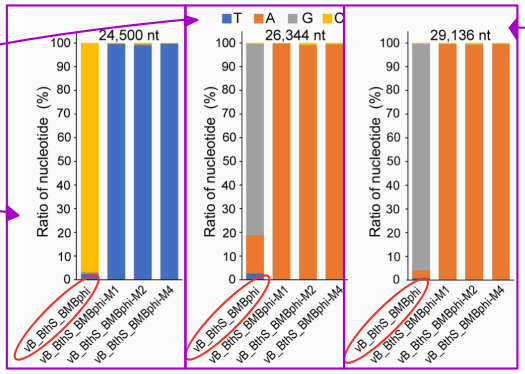
Figure 4. Analysis of the mutations of the phage-resistant bacterial mutants. (**A**) The mutant sites in four phage-resistant bacterial mutants. The functions and locations of the cell surface proteins that mutated in all the four mutants are indicated. (**B**) Alignment of the protein FlhA from the strains BMB171 and four phage-resistant mutants. The secondary structures of the protein are indicated. (**C**) Transmembrane domain of protein FlhA from strain BMB171 and PRB-1.

Mutation @26,344th nucleotide position

One of the conserved mutation sites in all regained-infectivity phage mutants was the mutation of one guanylate to adenylate in the gene encoding for tail protein Gp46.

Mutation @24,551th nucleotide position

One of the conserved mutation sites in all regained-infectivity phage mutants was the mutation of one cytidylate (C) nucleotide at 24,551th position in the genome to thymidine (T). This mutation was located in the phage baseplate protein (Gp44).



Mutation @29,136th nucleotide position

One of the conserved mutation sites in all regained-infectivity phage mutants was the mutation of one guanylate to adenylate in the gene encoding for distal tail protein Gp47.

Figure 5. Polymorphism of the mutant nucleotides in phage genome. The ratio of the nucleotide compositions of the three mutant nucleotides in four phage genomes is shown. The nucleotide composition was obtained by analyzing the raw reads archived by genome sequencing and the sites of each mutant nucleotides were shown.