

Treating multidrug and phage-resistant *Enterococcus* spp. by phage cocktails, endolysin, and bacteriocin

Mohamed El-Telbany^{1,2}, Chen Wang¹, Ayman El-Shibiny³, Yoshimitsu Masuda¹, Ken-ichi Honjoh¹, Takahisa Miyamoto¹

¹Graduate School Bioresource and Bioenvironmental Science, Kyushu University, Japan

²Department of Microbiology and Botany, Faculty of Science, Zagazig University, Egypt

³Biomedical Science, Zewail city of Science and Technology, Giza, Egypt



九州大学
KYUSHU UNIVERSITY

Background and significance

- ▶ *E. faecalis* causes life-threatening diseases like endocarditis. Bacteriophages have emerged as an alternative to antibiotics. Endolysins possess many pros such as a wide host range, and a low possibility of bacterial resistance.
- ▶ Here, we isolated and characterized a phage named vB_EfKS5. The antibacterial and antibiofilm activity of phage individually and in a cocktail were tested. The phage-resistant mutants were treated with endolysin LysEf-9 or/ and bacteriocin.

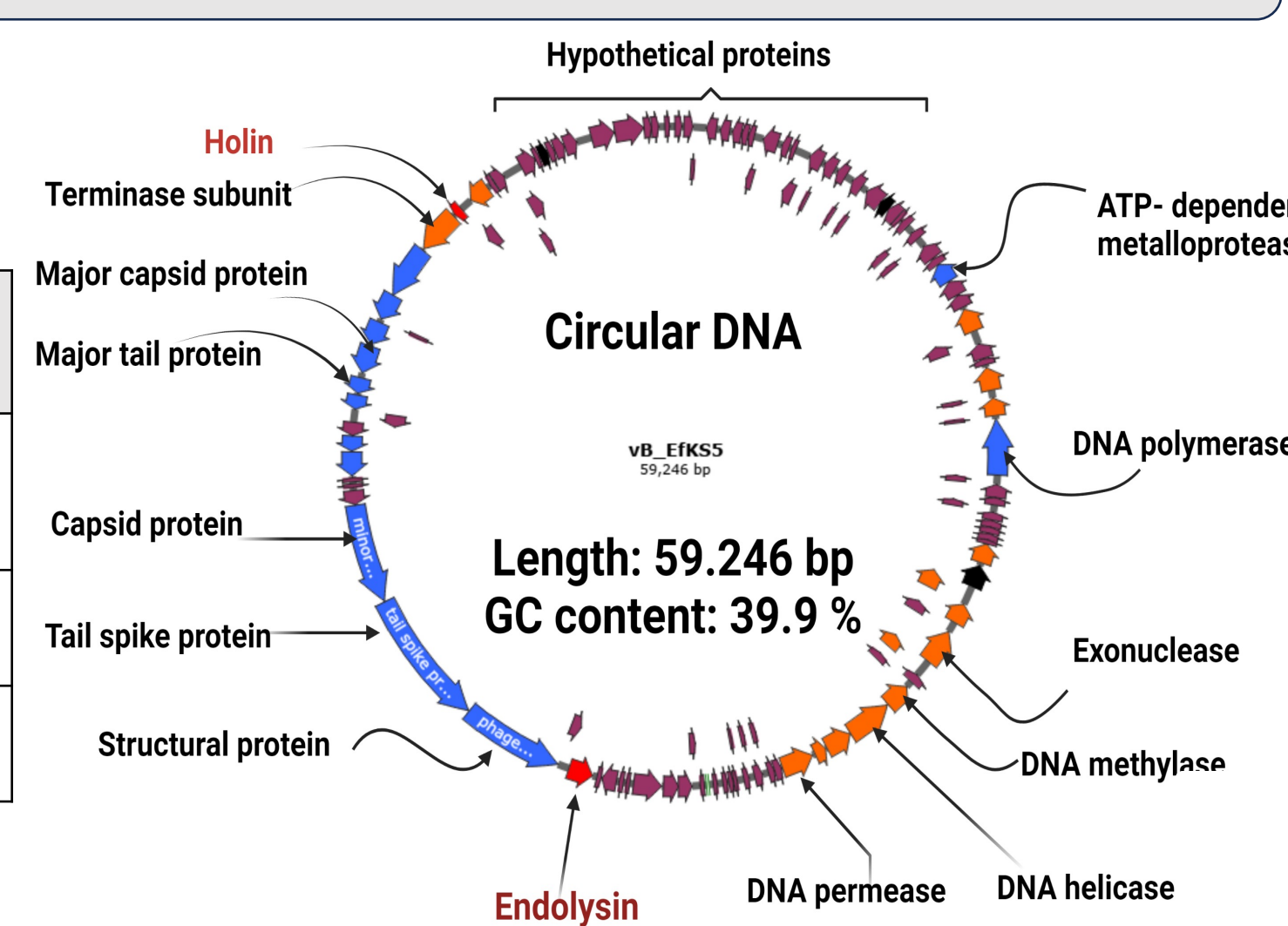
Conclusion

- ▶ Phage vB_EfKS5 is a lytic phage with a broad host range.
- ▶ Phage cocktail (vB_EfKS5 & PEF9) inhibited the biofilm formation of *E. faecalis* more than individual phage.
- ▶ The endolysin LysEf-9 from phage PEF9 at 62.5 µg/ml reduced the turbidity and viability of *E. faecalis*.
- ▶ Bacteriocin showed strong antibacterial activity.
- ▶ Combined endolysin LysEf-9 and bacteriocin showed the best effect against the phage-resistant mutants.

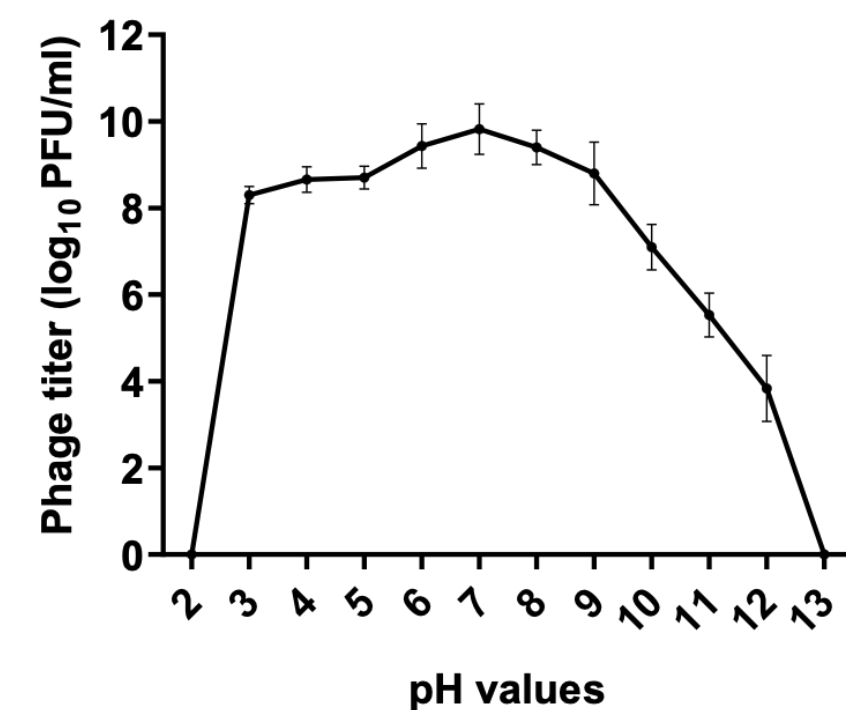
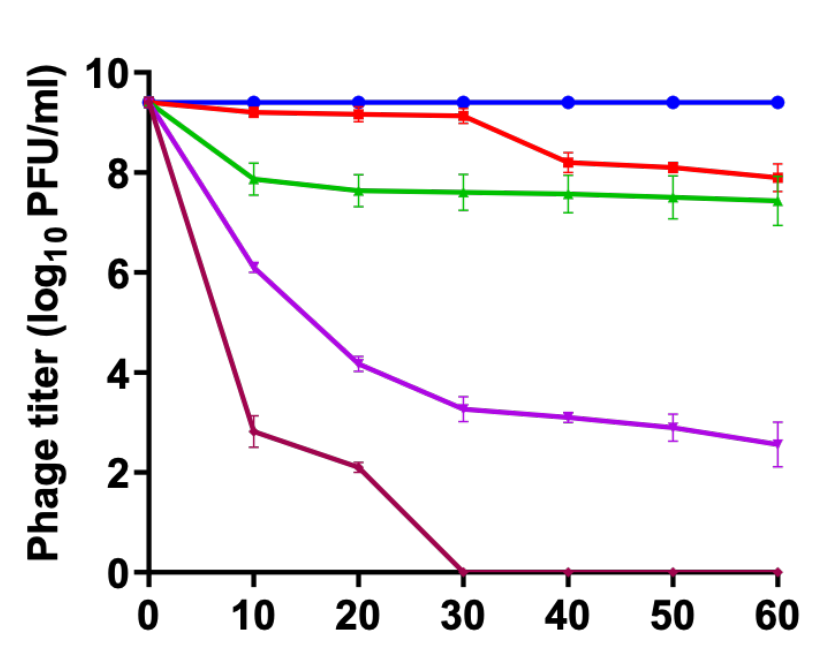
Results

Isolation and characterization of phage vB_EfKS5

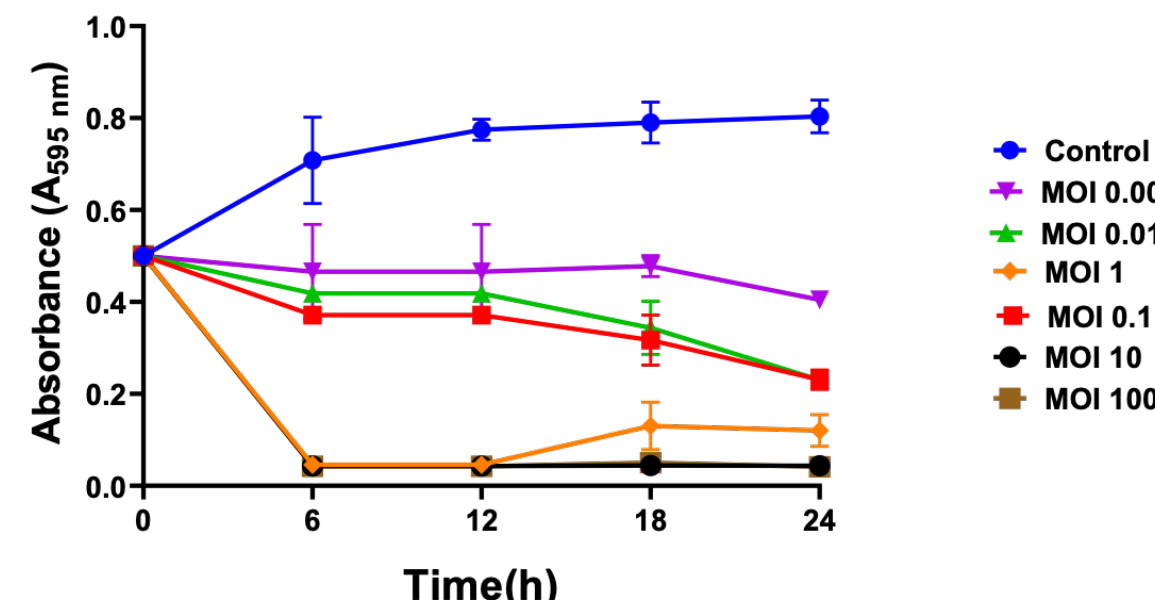
Bacteria (Hosts)	Bacteriophages				
	vB_EfKS 1	vB_EfKS 2	vB_EfKS 3	vB_EfKS 4	vB_EfKS 5
<i>E. faecalis</i>	15/28	9/28	11/28	17/28	22/28
<i>E. faecium</i>	5/6	1/6	6/6	3/6	2/6



Lysis profile analysis of isolated phages



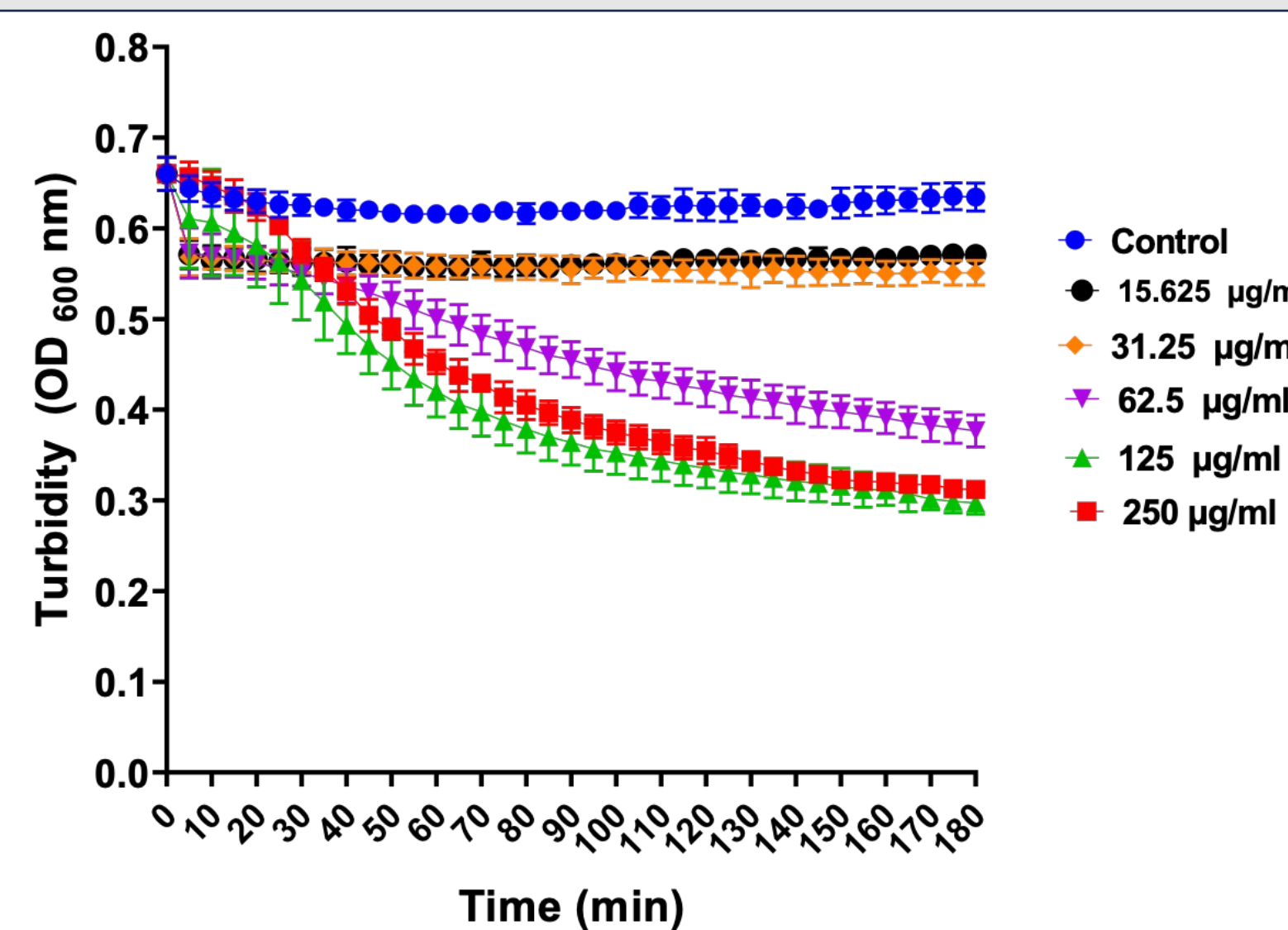
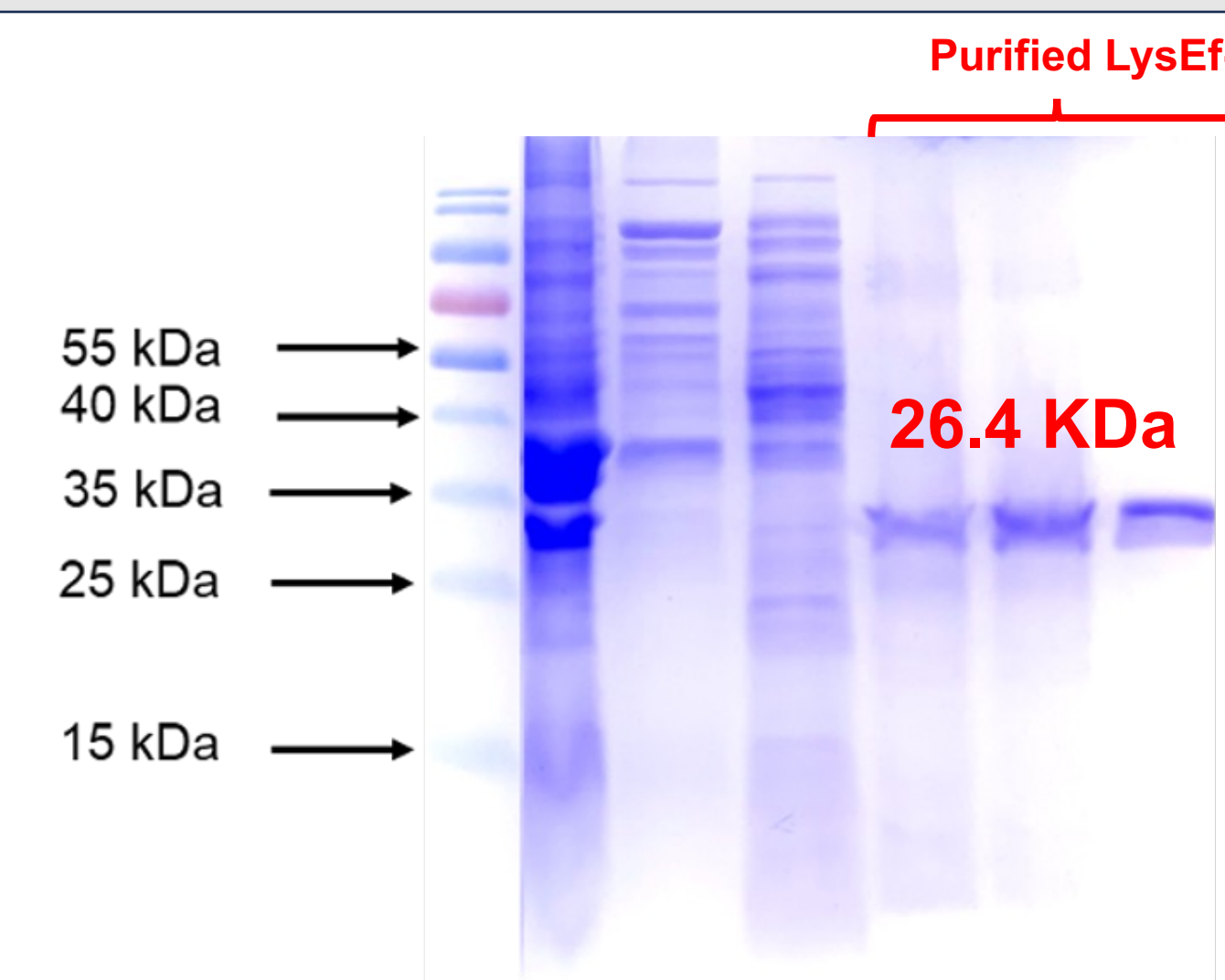
Phage vB_EfKS5 genomic map



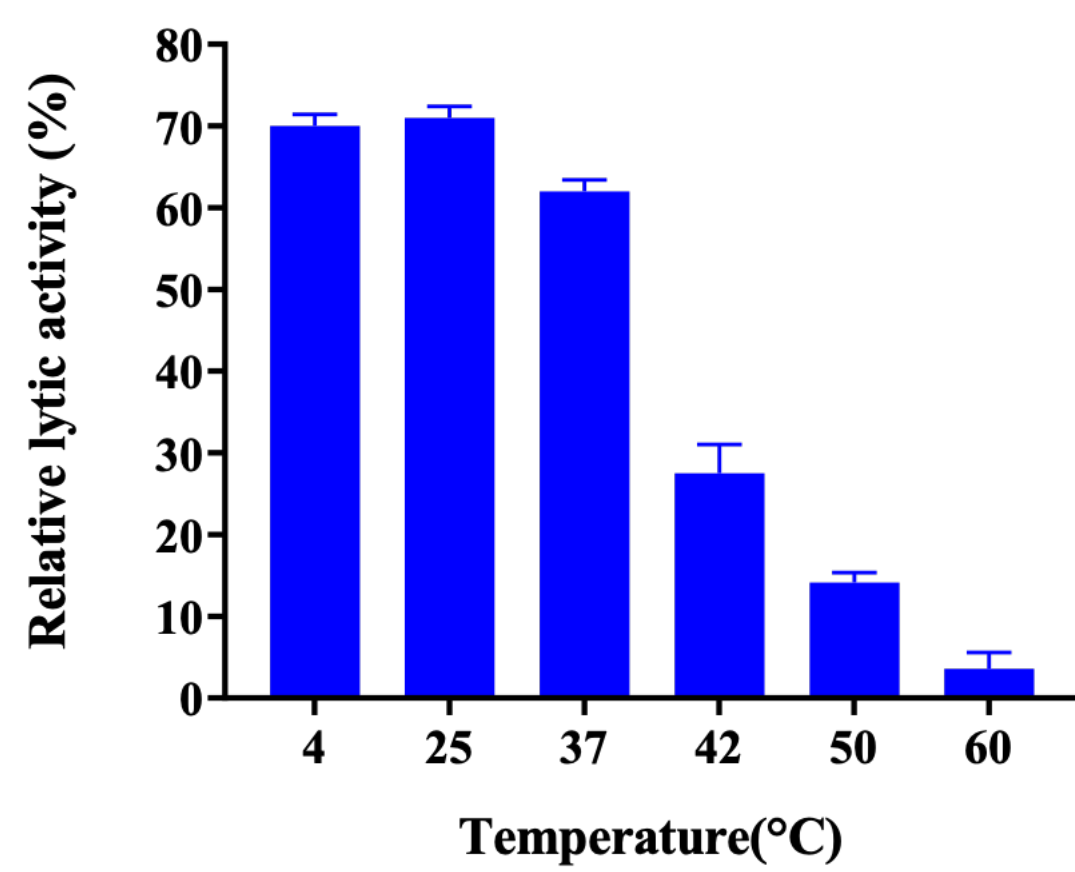
Temperature and pH stability

Bacterial killing activity

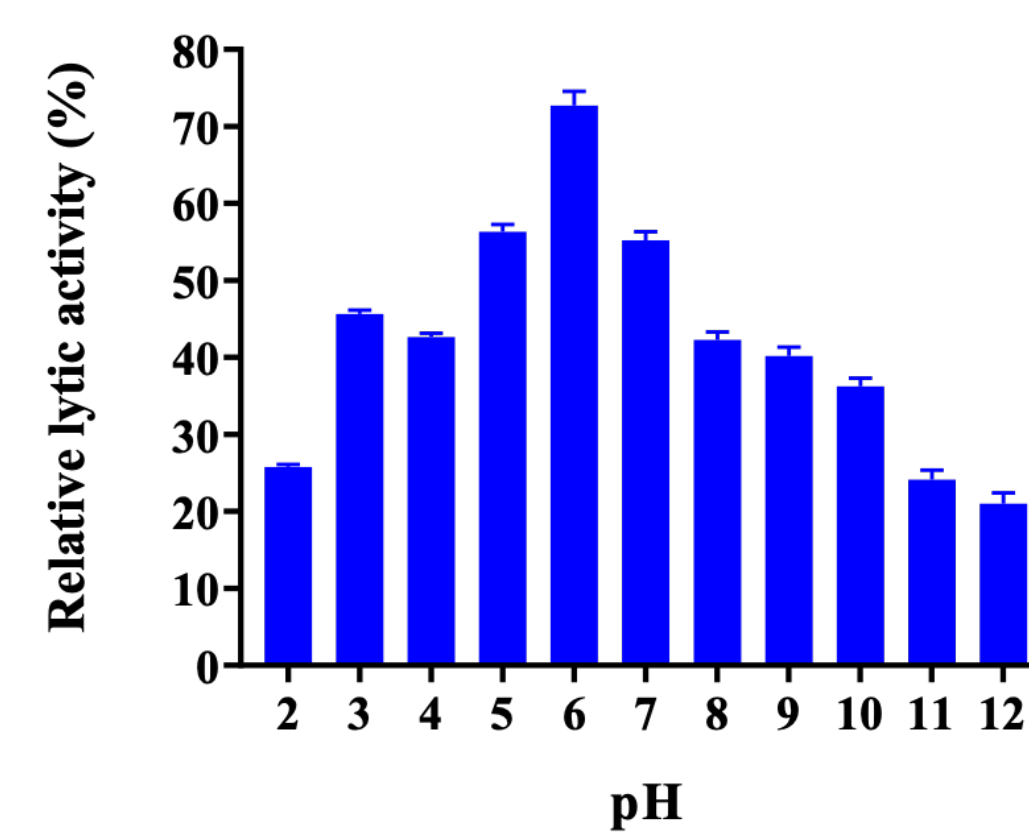
Characterization Endolysin LysEf-9 amplified from phage PEF9



Turbidity reduction of *E. faecalis* JCM 7783



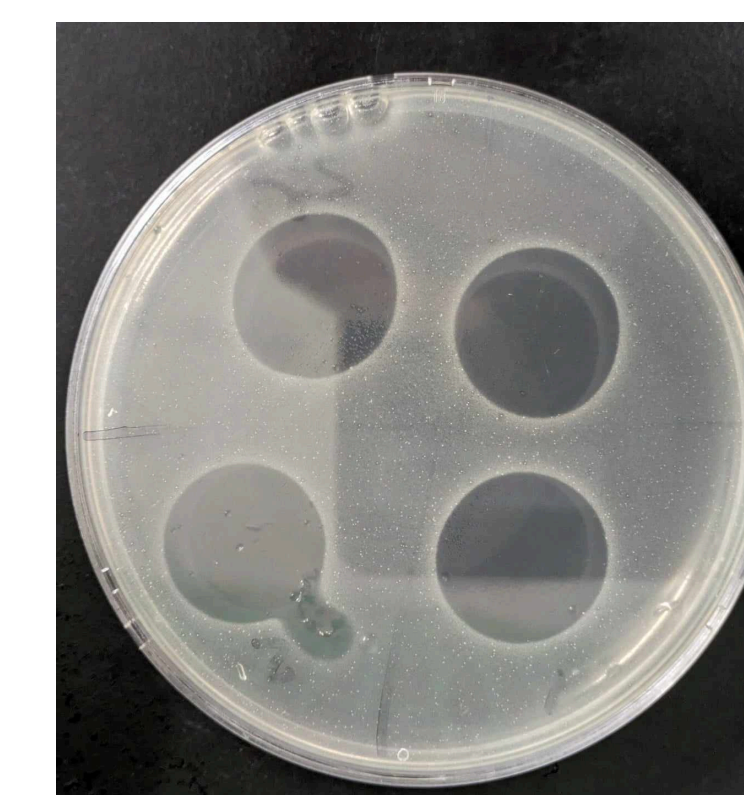
Temperature and pH stability



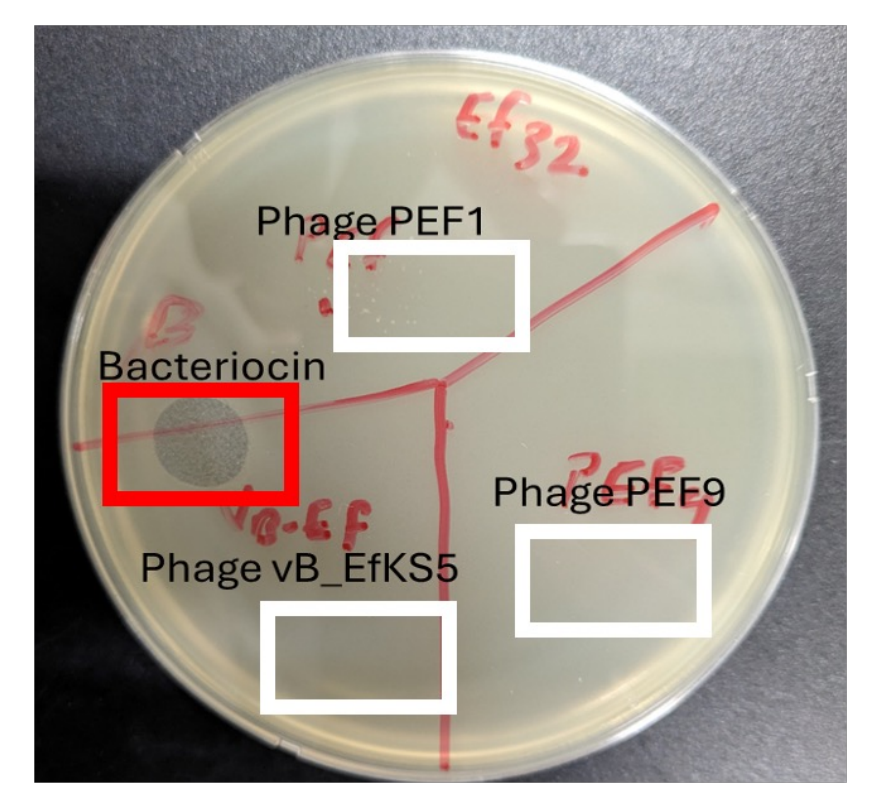
Isolation and activity of bacteriocin



Over-layer method

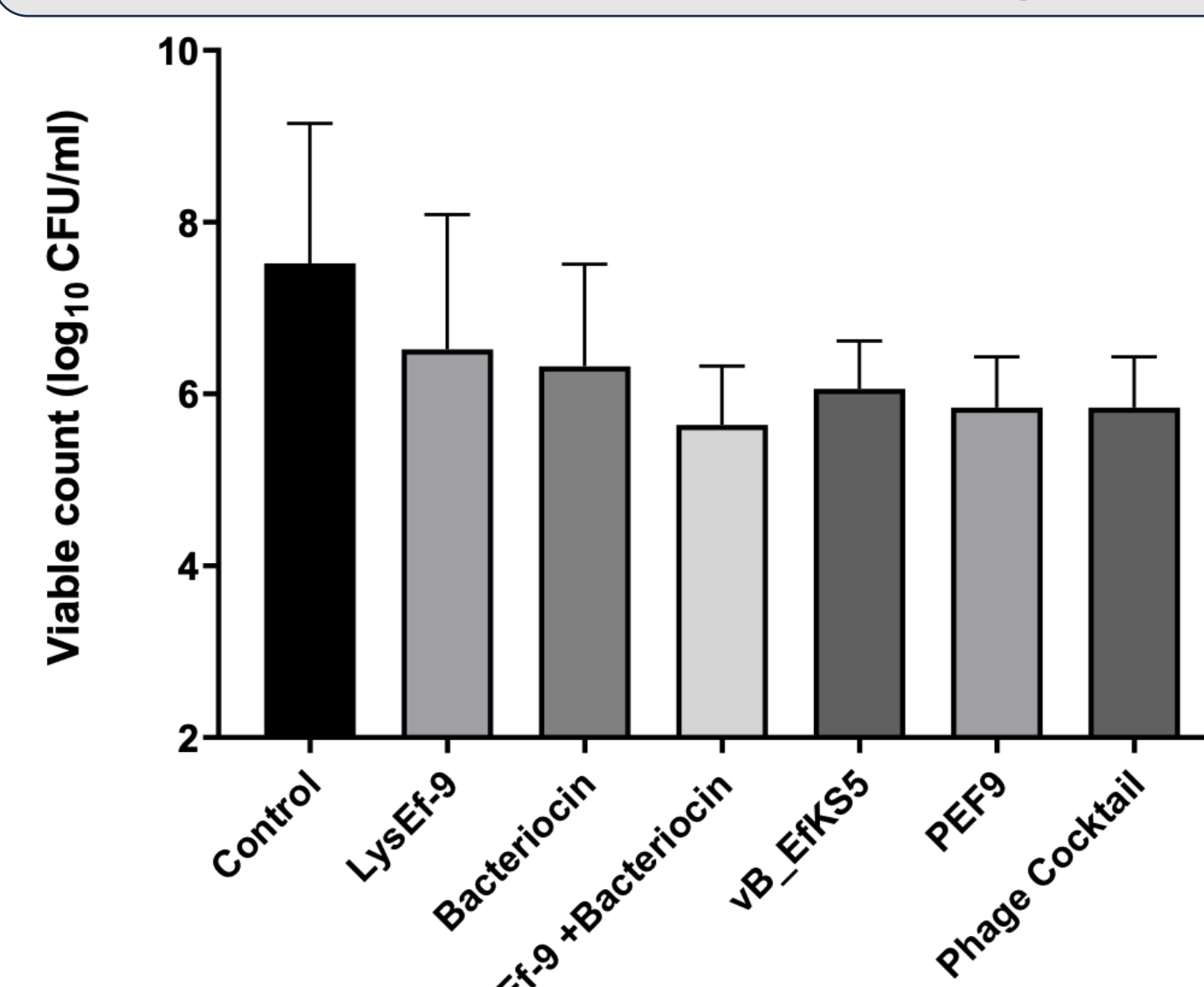


Spot-on-lawn- method

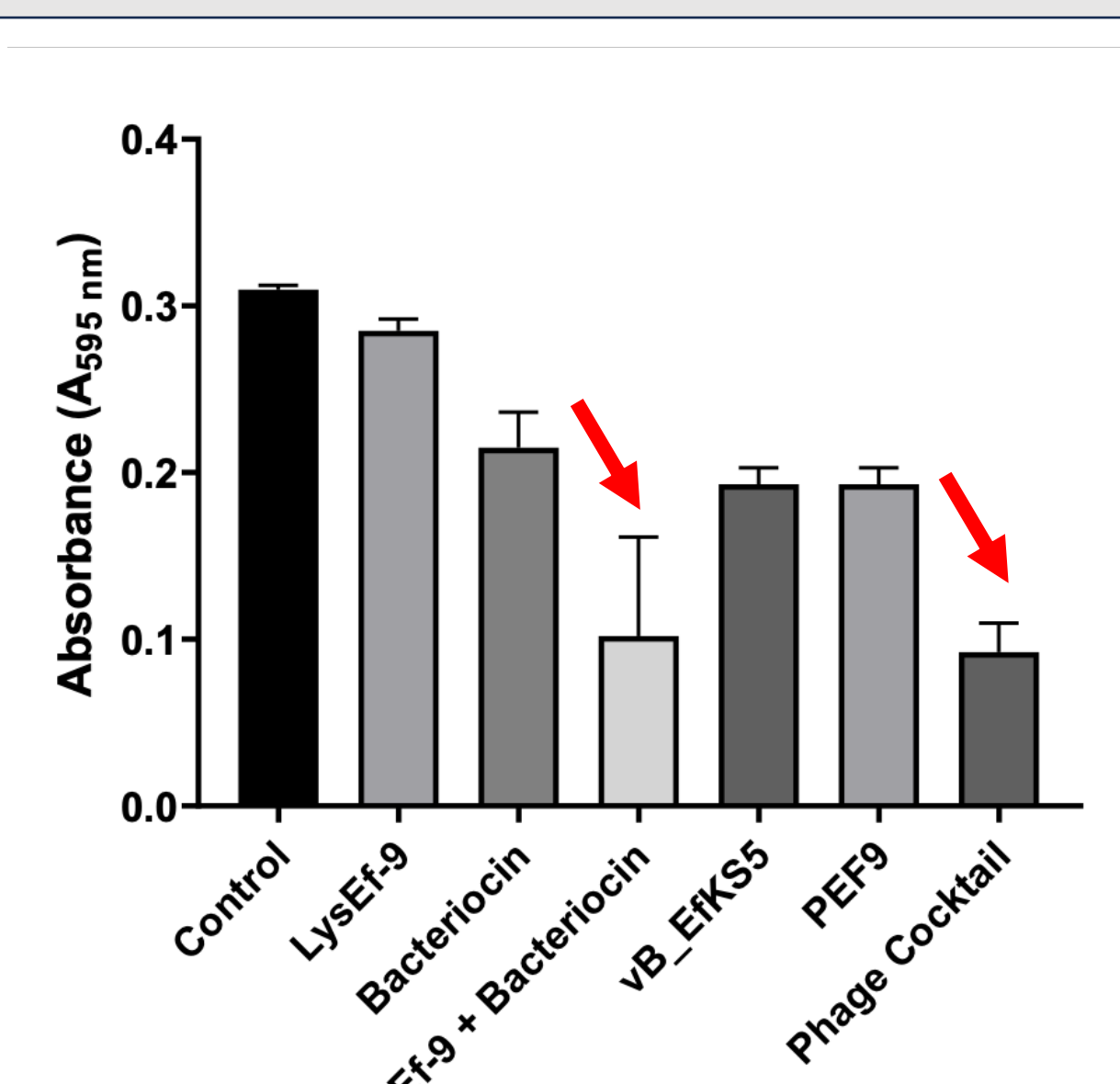


lytic activity against phage-resistant mutant

Effect of phage cocktail, LysEf-9, and bacteriocin on the growth and biofilm of multi-drug-resistant *E. faecalis* JCM 7783

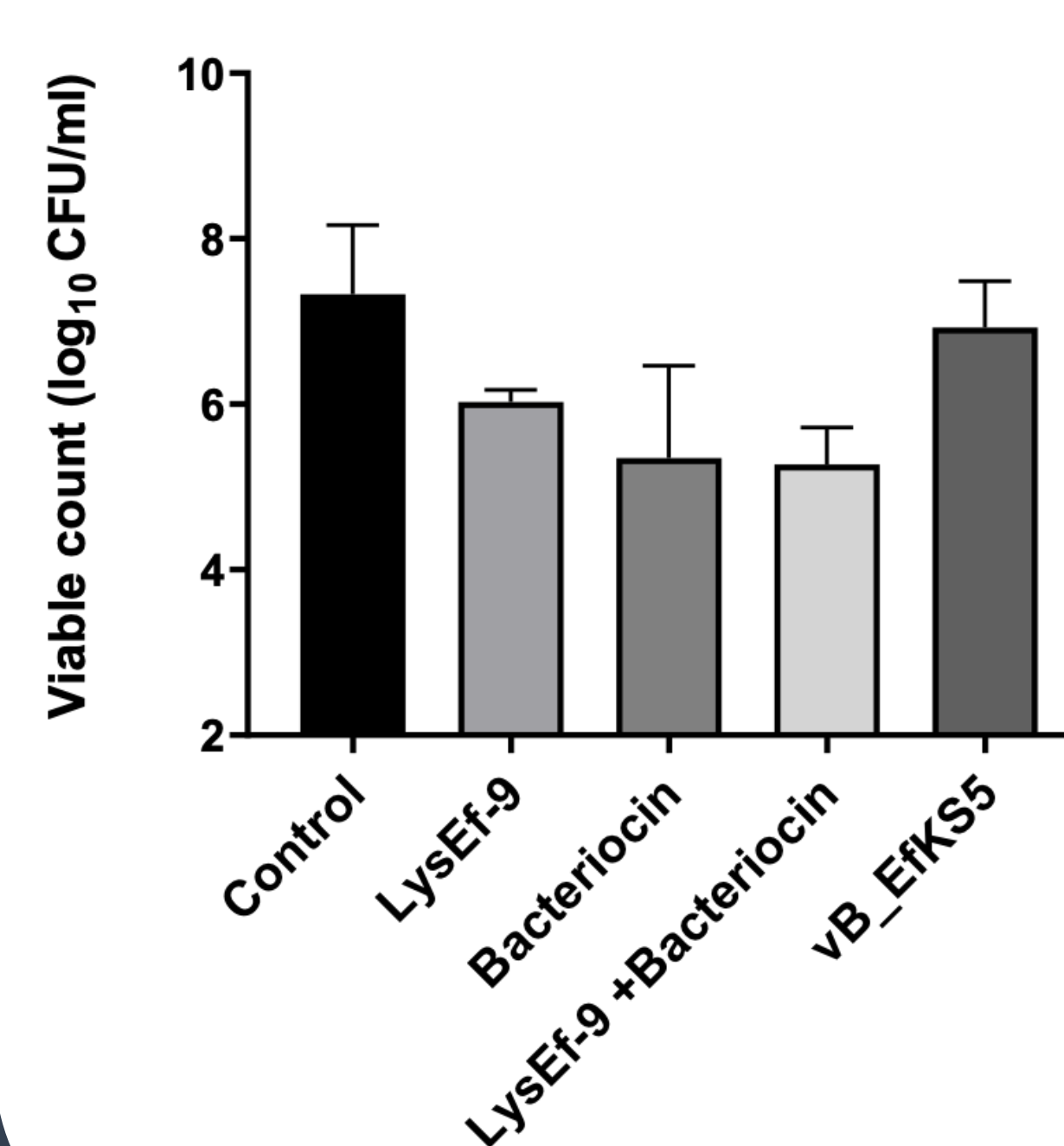


Reduction in planktonic growth after 24h

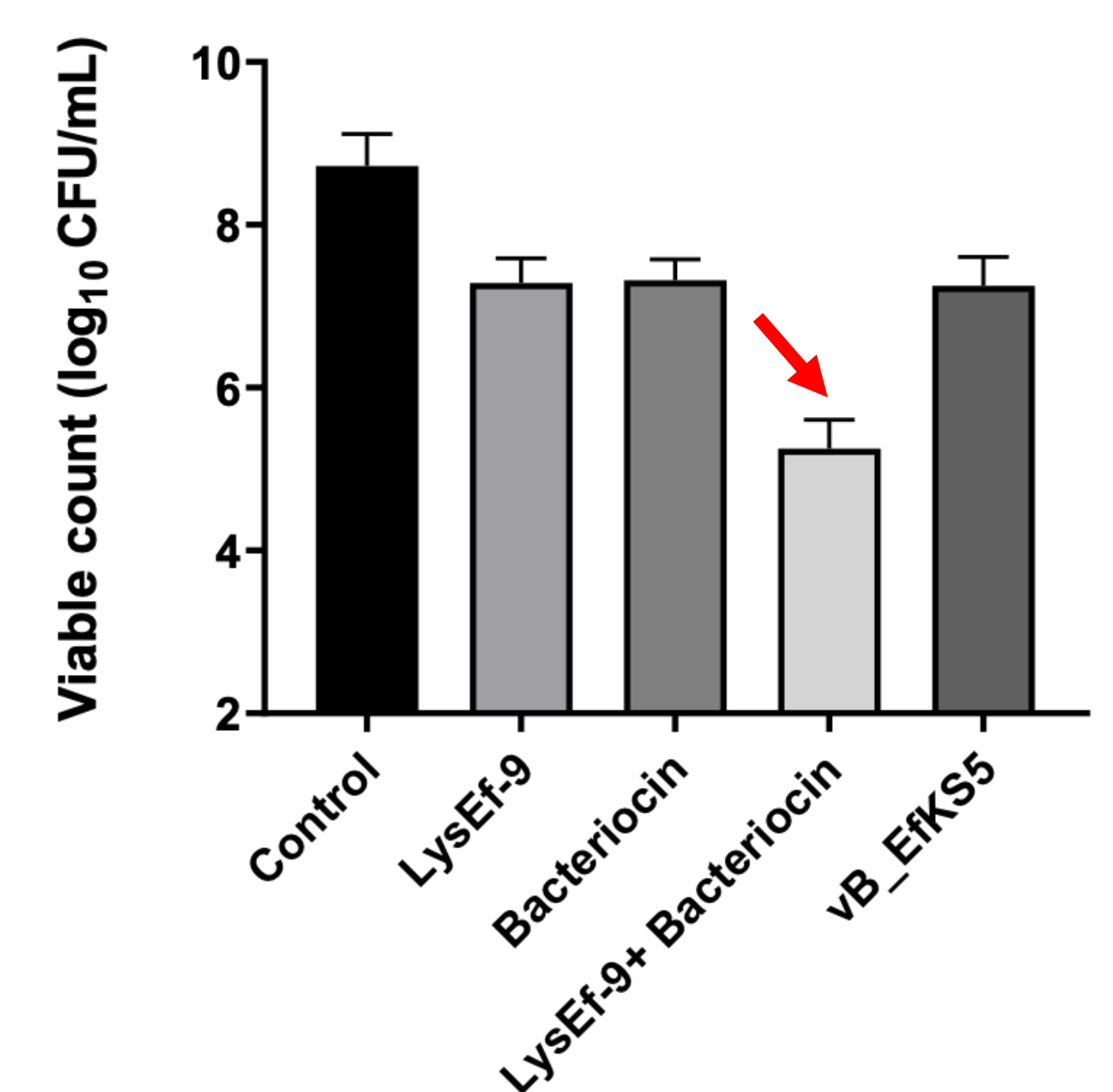


Inhibition of biofilm formation

Effect of endolysin LysEf-9 or/ and bacteriocin on the growth and biofilm of phage vB_EfKS5 resistant mutant



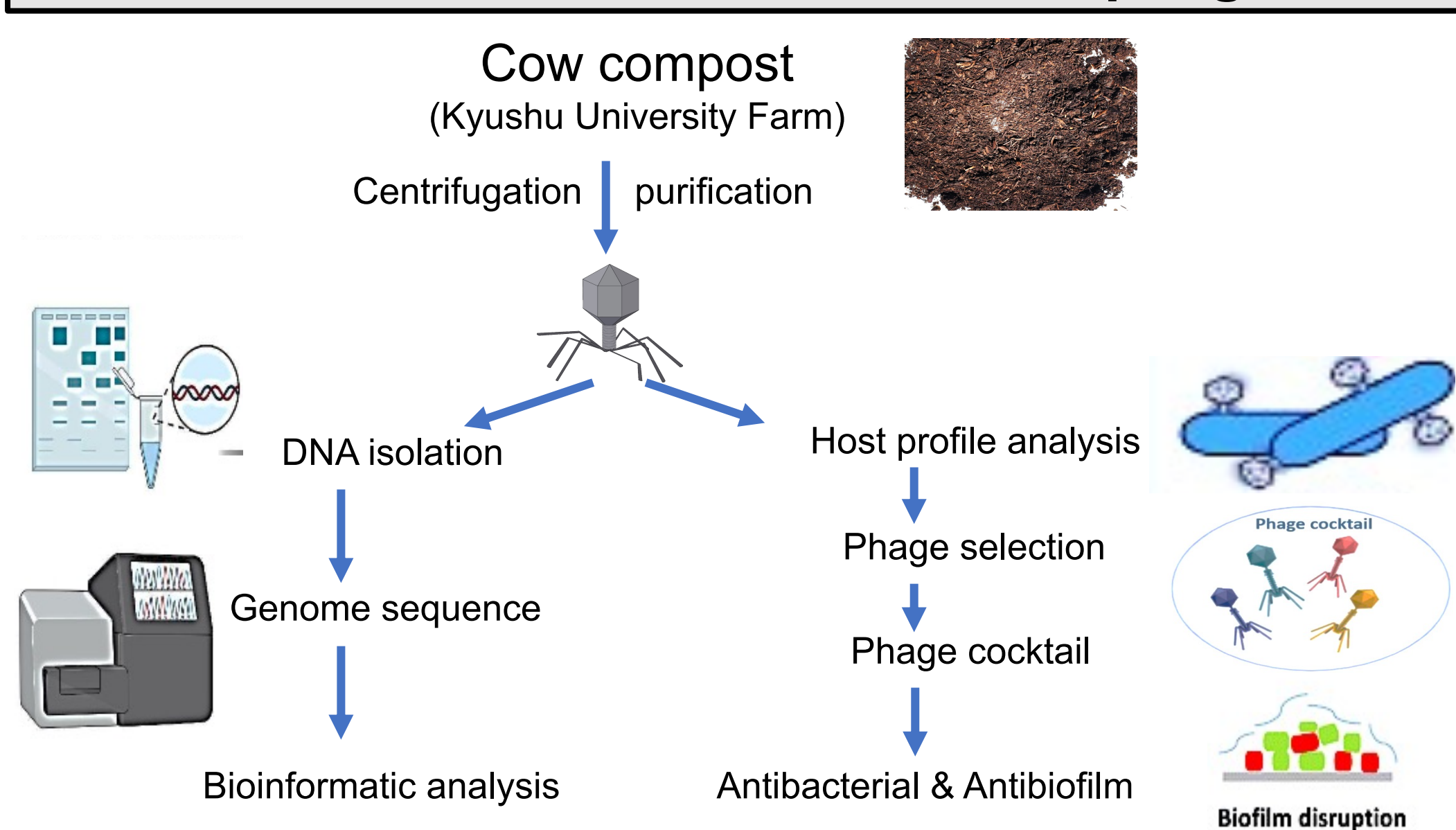
Reduction in planktonic growth after 24h



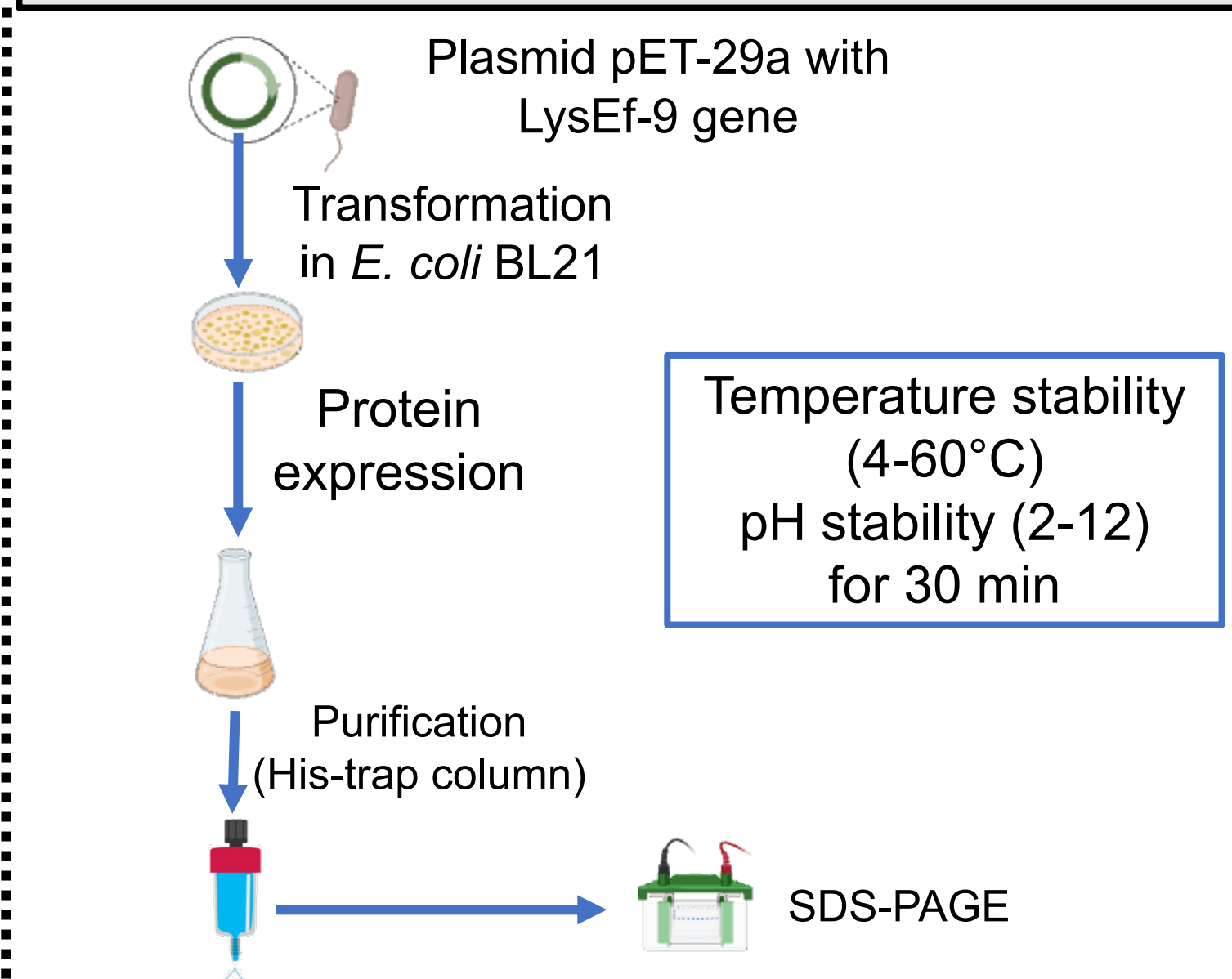
Removal of biofilm

Materials and methods

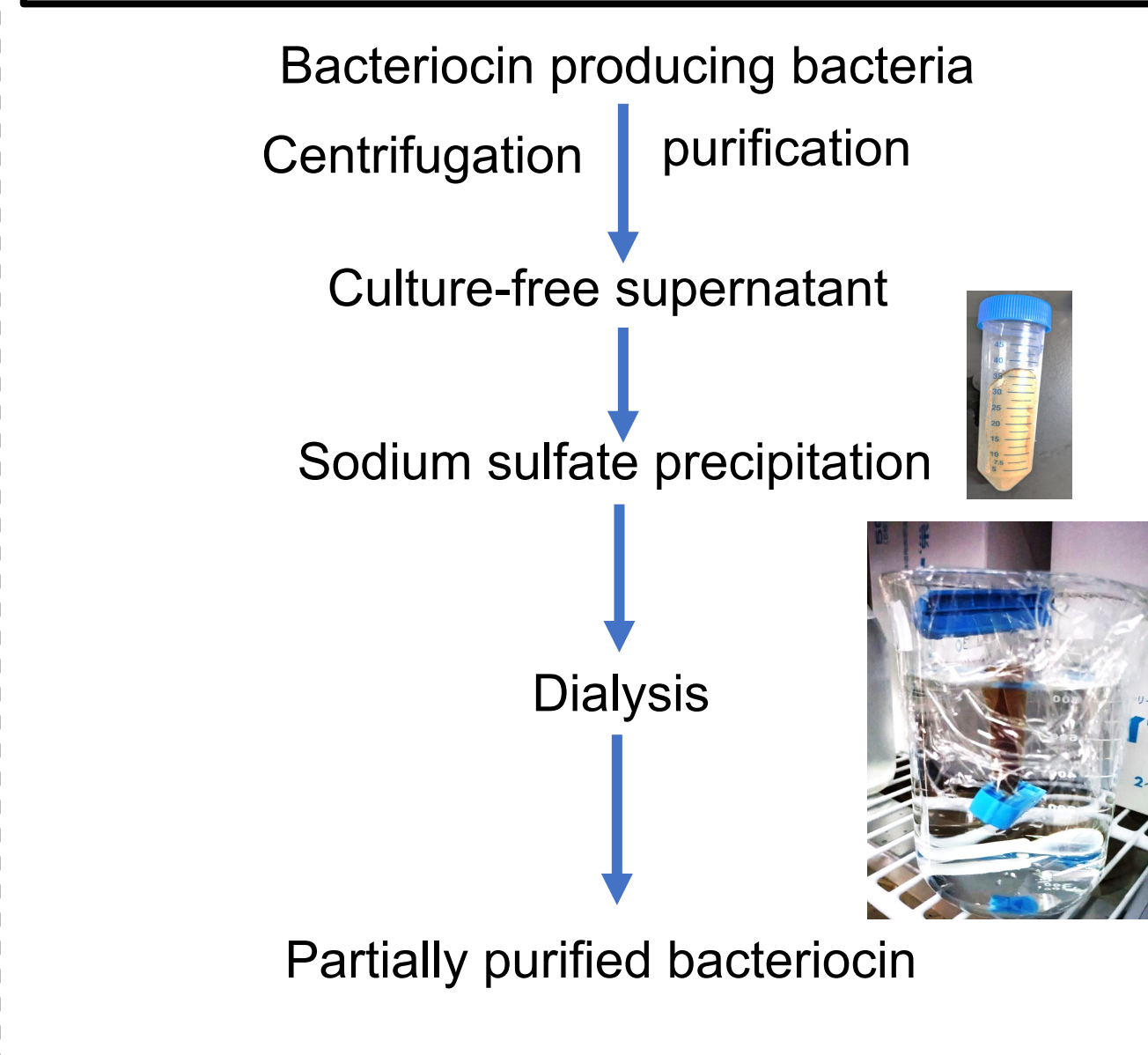
Isolation and characterization of phages



Expression and purification of Endolysin



Partially Purification of bacteriocin



Antibacterial and antibiofilm

