

Lytic Phage for Foodborne Pathogens Detection  
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Foodborne pathogens are among the most significant problems in maintaining the health of the population. The leading causes of foodborne illnesses in the United States are *Salmonella* and *Shigella* (1). *Staphylococcus aureus* is among top five pathogens contributing to domestically acquired foodborne illnesses. Since foodborne infections have a dramatic impact on morbidity and mortality, particularly of infants and children, timely detection of these pathogens is highly important. One of the promising approaches for detection of pathogenic bacteria in environment is the use of lytic phage biosensors.

We isolated lytic phage against *Staphylococcus aureus* with wide spectrum of hosts, including MRSA (Fig.1 A). Comparison of isolated phage's lytic activity with activity of *S. aureus* phage from the American Type Culture Collection showed that only isolated phage 12600 was effective against all tested MRSA strains (Fig.1 B).

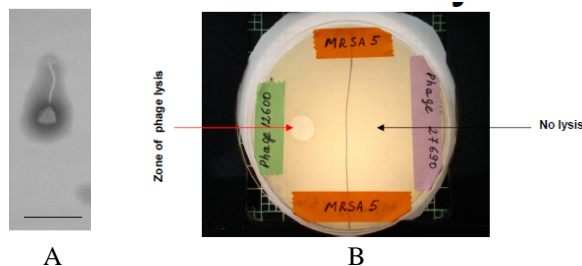


Figure 1. *S. aureus* phage: A – Transmission electron micrographs of isolated phage (bar – 200 nm); B – phage 12600 lytic activity against MRSA (left); phage 27690 has no lytic activity (right)

Co-cultivation of phage 12600 with MRSA resulted in quick lysis of bacterial culture (Fig. 2A). This phage, adsorbed on the gold surface remains its lytic activity (Fig. 2B).

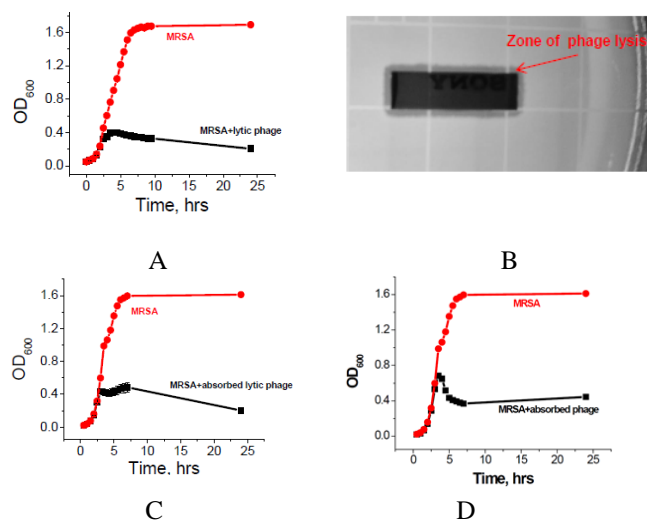


Figure 2. Lysis of MRSA by *S. aureus* phage 12600: A- co-cultivation with free phage; B and C – lysis by gold-absorbed phage; D – lysis by absorbed phage after storage

Co-cultivation of MRSA strain with adsorbed phage also resulted in lysis of bacterial culture. The kinetic of bacterial lysis was the same as with free phage (Fig. 2C). Immobilized phage remains alive and active after 6 days of storage at 4°C (Fig. 2D). Obtained results show that adsorbed phage can recognize, capture and lyse MRSA cells. This observation opens perspectives for fabrication of lytic phage biosensors for detection of pathogenic bacteria.

Next step in our research was isolation of phage for detection of *Salmonella* and *Shigella* pathogens. Newly isolated phage had unique spectrum of lytic activity – it was effective against all 23 tested strains of *Salmonella* spp., 2 strains of *Shigella* spp. and showed no activity against other closely related bacteria (Fig. 3; Table 1).

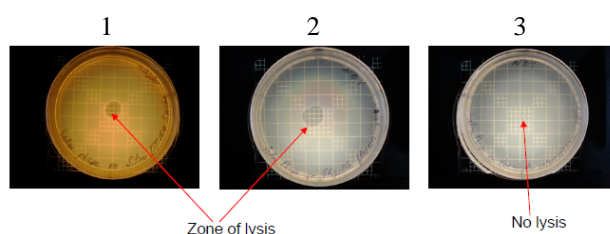


Figure 3. Selectivity of *Salmonella* phage: 1 - *Salmonella typhimurium* DT 104 2- *Shigella flexneri*; 3 - *Yersinia enterocolitica*

Bacterial cultures, sensitive to <i>Salmonella</i> phage	Bacterial cultures, resistant to <i>Salmonella</i> phage
1. <i>S. typhimurium</i> Health 9491	1. <i>Pseudomonas aeruginosa</i>
2. <i>S. typhimurium</i> DT 104 Dalry	2. <i>Pseudomonas fluorescens</i>
3. <i>S. diarisonae</i>	3. <i>Escherichia coli</i> ATCC 11775
4. <i>S. panama</i> SA 3583	4. <i>Klebsiella pneumoniae</i> 13882
5. <i>S. indica</i> SA 4401	5. <i>Yersinia enterocolitica</i>
6. <i>S. derby</i> SARB 10	6. <i>Proteus mirabilis</i>
7. <i>S. typhimurium</i> LT2	7. <i>Staphylococcus aureus</i> ATCC 12600
8. <i>S. mission</i>	8. <i>S. aureus</i> ATCC 27690
9. <i>S. montevideo</i>	9. <i>S. aureus</i> 10292
10. <i>S. typhimurium</i> 6787	10. <i>S. aureus</i> 10497
11. <i>S. typhimurium</i> Health 1390	11. <i>S. aureus</i> 10686
12. <i>S. bongori</i> SA 4910	12. <i>S. aureus</i> MRSA 1
13. <i>S. typhimurium</i> Nal 1x fecal	13. <i>S. aureus</i> MRSA 2
14. <i>S. minnesota</i>	14. <i>S. aureus</i> MRSA 5
15. <i>S. salamae</i> SA 41106	15. <i>S. aureus</i> MRSA 13
16. <i>S. typhimurium</i> 520-96	16. <i>S. aureus</i> MRSA 26
17. <i>S. Thompson</i> 265-4	17. <i>S. aureus</i> MRSA 34
18. <i>S. infantis</i> SARR 27	18. <i>S. aureus</i> MRSA 45
19. <i>S. paratyphimurium</i>	19. <i>Bacillus anthracis</i> Sterne
20. <i>S. typhimurium</i> DT 104 Swine	20. <i>R. subtilis</i> ATCC 6051
21. <i>S. dublin</i> SA 2424	
22. <i>S. typhimurium</i> 9683	
23. <i>S. typhimurium</i> ATCC: 13311	
24. <i>Shigella flexneri</i>	
25. <i>Shigella sonnei</i>	

Table 1. Bacterial test-cultures for detection of *Salmonella* phage selectivity

Strong bacterial specificity of isolated phages makes it possible to create biosensors with near-to-real-time capacity in recognition of foodborne pathogens.

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