The Isolation and Typing of Coliphages Specific to Various Wild-Type *Escherichia coli* Strains and Phage-Resistant Mutants

Author: Christe Marbbn

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Abstract **Phage-resistant *Escherichia coli* mutants are typically achieved via the inoculation of infectious coliphages. The aim of this study was to assess the host-range of an assortment of coliphages with various strains of wild-type *E. coli* and to test the degree of sensitivity or resistance of the isolated mutants. For this purpose, coliphages were prepared from sewage influent and cultivated using six distinct strains of *E. coli* to observe for the formation of plaque. It was found that the addition of certain coliphages induced phage-resistant colonies of mutant *E. coli* within the plaque formed. Under further examination, mutant colonies were also resistant to the six coliphages tested. Coliphages ΦW3104 and ΦC possessed the widest host range as they were able to infect five out of the six strains of *E. coli*. It is possible that the phage-resistant mutants carry a deletion or mutation in a gene coding for bacteriophage receptor components. This study demonstrates that somatic coliphages are a promising tool for investigating mutagenesis in *E. coli*.**

Introduction

Among the many systems available for studying mutation, the gram-negative bacterium *E. coli* has long been used in research due to its tendency to mutate under particularly stressful conditions and become resistant to antimicrobial agents. A variety of different *E. coli* strains have been characterized to show considerable differences in their physiology and overall functioning (Hattori *et al.,* 2008). These differences are mainly caused by genetic variation resulting from the natural biological process of mutation. Like higher species, *E. coli* are susceptible to infection. During transduction in horizontal gene transfer, bacterial viruses, known as bacteriophages, infect the bacterium by introducing a small amount of viral genome. In turn, this may cause the bacterium to lyse and spread its destructive phage genome (Alavidze *et al.,* 2001). Since bacteriophages are bacteria-specific, host specificity is used to differentiate different strains of individual species of bacteria.

Coliphages are viruses that primarily infect *E. coli*. They consist of a capsid that contains single or double stranded DNA and, in optimal conditions, may lyse the host cell in 20 to 30 minutes (Jofre *et al.,* 2003) upon binding to their appropriate receptor. One variety of coliphage are called male-specific (F+) because they infect their host via the F-pilus of male *E. coli* strains. When coliphages infect the host cell via the bacterial cell wall, they are referred to as somatic coliphages. Unlike the male-specific coliphages which do not replicate outside the human body, the somatic coliphages replicate in any environment, and thus are frequently found in greater abundance (EPA, 2001). In a study conducted by Alum *et al.,* (2005) assessing water quality in the United States–Mexican border region, somatic coliphages were greater by one order of magnitude compared to F+ coliphages. Coliphage detection methods are simple, fast, and relatively inexpensive to perform (EPA, 2001).

The goal of the following investigation is to isolate phages from sewage waste that are specific to wild type *E. coli* strains and assess the host range of each phage culture with regards to their ability to lyse the cells by the visible formation of plaque. In addition, this study focuses on the transition of *E. coli* strains from being phage susceptible to being phage resistant.

Materials & Methods

Isolation of Coliphages from Sewage: A volume of 1 mL liquid culture of *E. coli* strain K12 (Ward's) was added to 4 mL of prepared nutrient broth (Sigma-Aldrich). Raw sewage collected from a wastewater treatment plant (Oshawa) was added to the mixture and incubated at 37ºC for 24-hours under agitation to observe for cell lysis. The same bacterial strain was re-inoculated with the crude phage solution and allowed to further multiply. The mixture was centrifuged, filtered through a 0.2 µm filter, and stored at 4ºC.

Determination of Bacteriophage Titer: Eight serial ten-fold dilutions of the phage were performed using saline as dilutant. A volume of 0.3 mL of the host bacterial culture used to enrich the coliphage and 0.1 mL of undiluted enriched coliphage solution was added to a tube of soft agar (Sigma-Aldrich) warmed to 55ºC. The mixture was poured onto a plate of nutrient agar (Sigma-Aldrich) and incubated for 24-hours at 37ºC. This procedure was repeated with the 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8} dilutions to observe for the formation of plaque.

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Phage Typing and Isolation of Phage Resistant Mutants: A volume of 0.1 mL of *E. coli* strains K12, R10, R12, W3104, B, and C (Ward's) were transferred onto separate nutrient agar plates. Coliphage isolates were spotted in their respective portions on each of the plates and incubated at 37ºC for 24-hours to observe for spontaneous growth of phage-resistant mutant *E. coli*. A single colony of mutant bacteria was streaked on a nutrient agar plate and allowed to grow; this procedure was repeated for two other potential mutants. Moreover, liquid cultures of the three spontaneous mutants were grown at 37ºC for 24-hours in nutrient broth. Phage sensitivity of phage-resistant mutants was tested by spotting 5 µL of each coliphage onto the three isolated mutant *E. coli* strains and incubated at 37ºC for 24-hours to observe for the formation of plaque.

Results

Phage Infection of E. coli and the Determination of Titer: The *E. coli* K12 strain appeared to be fully resistant to bacteriophages present within the raw sewage in which it was incubated, as no plaque had formed (data not shown). Likewise, since *E. coli* K12 remained resistant to all five of the varying concentrations of the enriched coliphage (data not shown), a host-specific bacteriophage associated with K12 is not present.

Table 1. The susceptibility of six distinct wild-type *E. coli* strains to different coliphages (Top), and the susceptibility of phage-resistant *E. coli* mutants against the six phages (Bottom).

E. coli Strain	Phage Type					
	ΦK12	ΦR10	ΦR12	ΦW3104	ΦB	ΦC
K12	–	–	–	[+]	–	[+]
R10	–	+	–	[+]	–	+
R12	[+]	–	[+]	[+]	–	[+]
W3104	[+]	[+]	[+]	[+]	[+]	[+]
B	+	[+]	+	–	[+]	+
C	[+]	[+]	+	[+]	[+]	[+]
Mutant *E. coli* Strain						
C	–	–	–	–	–	–
R12	–	–	–	–	–	–
W3104	–	–	–	–	–	–

(+) Plaque formation
([+]) Plaque formation with mutant colonies of bacteria
(–) No Plaque formation

Susceptibility of E. coli and Potential Mutants to Different Phages: The susceptibility of *E. coli* to different phage isolates was analyzed by a phage spot test (Table 1). Generally, host range varied for each coliphage. Coliphages ΦW3104 and ΦC showed the most extended host range, indicating a lack of resistance among all six *E. coli* strains by these species. The formation of bacterial colonies within the phage-induced plaque potentially indicates that the infected *E. coli* strain has undergone spontaneous mutation and is no longer susceptible to the coliphage. In fact, the mutant colonies obtained from strain C, R12, and W3104 illustrate notable differences when spotted with the same assortment of phages; rather than undergoing lysis and forming plaque like their wild-type predecessors, they were fully resistant to coliphage attack. This demonstrates that the colonies obtained are true *E. coli* mutants.

Discussion

The relationship between infectious coliphages and *E. coli* is thoroughly established through the lytic cycle. The main objective of this study is to isolate coliphages from raw sewage that are specific to several different wild-type *E. coli* and to assess the host range of each phage in regards to their ability to undergo lytic infection by the visible formation of plaque. For the wild-type *E. coli* strain K12, no plaque or cell lysis was observed in any of the experiments conducted when inoculated with the raw sewage. This implies that the K12 strain of *E. coli* is either resistant to the coliphages present within the raw sewage or that the sewage did not contain a phage specific to K12. In literature, when *E. coli* strain K12 is mutated to produce an altered form of lipopolysaccharide, ΦK12 coliphages are capable of forming plaque after inoculation (Beher & Pugsley, 1980). Therefore, the coliphages may have failed to recognize the outer membrane components, which are either masked or inaccessible in the K12 host, and as a result, cell lysis did not occur (Beher & Pugsley, 1980). In fact, Pugsley and Schnaitman (1978) showed that when wild-type *E. coli* K12 is resistant to various phages, they appear to lack the outer receptor membrane OmpC protein. This may also help to explain why coliphage ΦR10, ΦR12, and ΦB did not form plaque, since other coliphages also use this protein in the constitution of their receptor (Beher & Pugsley, 1980). Since the K12 coliphage formed plaque on the other strains examined, it confirms that the coliphage was, in fact, present after enrichment.

The two most versatile and widespread coliphages examined in this experiment were ΦW3104 and ΦC, since five out of the six *E. coli* strains tested efficiently adsorbed the phages. The main reason why these two phages have an extended host range is because most wild-type *E. coli* strains possess a single receptor protein LamB that facilitates the coliphages ΦW3104 and ΦC adsorption (Ludwig *et al.,* 1984). Presumably, the *E. coli* strains which failed to lyse in the presence of these phages may lack the signals necessary to export and position LamB protein in the outer membrane (Ludwig *et al.,* 1984).

Newly formed bacterial colonies which grew from the phage-induced plaque formation on the bacterial lawns,

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appeared to be genuine mutants resistant to the phages used throughout the experiment. The emergence of phage-resistant cells has been noted to be due to spontaneous changes that occur in their outer membrane components responsible for specific phage binding (phage receptors). Alteration or deletion of phage receptors from the cell surface protects bacteria from phage attack (Hori *et al.,* 2003). In fact, Hattori *et al.,* (2008) demonstrated that when the *OmpC* gene is mutated or deleted, phage resistance is promoted.

Overall, this study confirms the underlying mechanism of coliphage infection and the mutagenic effects they induce in varying *E. coli* strains during replication and division, and provides a suitable foundation for further biochemical analysis regarding outer membrane phage receptor proteins.

References

Alavidze, Z., Glenn, M.J., & Sulakvelidze, A. (2001). Bacteriophage Therapy. *Antimicrobial Agents and Chemotherapy*, 45(3): 649-659.

Alum, A., Alvarez, M., Hodon, R., & Mendoza, J. An assessment of water quality and microbial risk in rio grande basin in the United States-Mexican border region. *Journal of Water and Health*, 3(2):209-218.

EPA: Environmental Protection Agency. (2001). USEPA Manual of Methods for Virology: Chapter 16. 600/4-84/013.

Hattori, K., Miyanaga, K., Suzuki, K., & Tanji, Y. (2008). Spontaneous Deletion of a 209 Kilobase-Pair Fragment from the *Escherichia coli* Genome Occurs with Acquisition of Resistance to an Assortment of Infectious Phages. *Applied and Environmental Microbiology*, 74(14): 4256–4263.

Hori, K., Miyanago, K., Shimada, T., Tanji, Y., Unno, H., & Yoichi, M. (2003). Toward rational control of *Escherichia coli* O157:H7 by a phage cocktail, *Applied Microbiology & Biotechnology*, 64(2): 270-274.

Jofre, J., Katayama, H., Mocé-Llivina, L., & Muniesa, M. (2003). Bacterial host strains that support replication of somatic coliphage. *Antonie van Leeuwenhoek*, 83(4): 305-315.

Ludwig R.A., Raymond C.K., & De Vries G. (1984). Extension of bacteriophage A host range: Selection, cloning, and characterization of a constitutive X receptor gene. *Proceedings of the National Academy of Science USA*, 81: 6080-6084.

Pugsley, A.P., & Schnaitman, C.A. (1978). Outer membrane proteins of Escherichia coli. VI. Evidence that bacteriophage-directed protein 2 functions as a pore. *Journal of Bacteriology*, 133:1181-1189.

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