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Mechanisms Responsible for a ΦX174 Mutant's Ability To Infect *Escherichia coli* by Phosphorylation[▽]

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The ability for a virus to expand its host range is dependent upon a successful mode of viral entry. As such, the host range of the well-studied ΦX174 bacteriophage is dictated by the presence of a particular lipopolysaccharide (LPS) on the bacterial surface. The mutant ΦX174 strain JACS-K, unlike its ancestor, is capable of infecting both its native host *Escherichia coli* C and *E. coli* K-12, which does not have the necessary LPS. The conversion of an alanine to a very reactive threonine on its virion surface was found to be responsible for the strain's expanded host range.

Critical to the success of any pathogen is its ability to both infect and persist within its host species. Extrapolation of the evolutionary mechanisms responsible for viral emergence has predominantly relied upon introducing the pathogen to susceptible hosts and/or introducing pathogen-specific receptors to the host species. A substantial effort has been conducted utilizing model bacteriophage species and their bacterial hosts, particularly ΦX174. As ΦX174 necessitates the presence of a particular lipopolysaccharide (LPS) on the bacterial surface, present for both native hosts *Escherichia coli* C and *Salmonella typhimurium* (17), several studies have utilized this two-host system to identify host-specific adaptations (6, 11, 21). Although enterobacteria without this surface protein are resistant to the ΦX174 wild type (19), previous studies have successfully infected mutant *E. coli* K-12 strains (10, 18) and wild-type *E. coli* K-12 by utilizing an intermediary mutant *E. coli* K-12 host species (3).

While wild-type ΦX174 is not capable of infecting wild-type *E. coli* K-12, several other phages are capable, most notably the lambda and lambda-like phages which infect *E. coli* K-12 through interaction with LamB, a cell surface receptor (20). For this to occur, oxidative phosphorylation is required for successful infection of *E. coli* K-12 by the lambda DNA (1). Thus, one can speculate that the expanded host range in the previous study using an intermediary host (3) can be the result of either the creation of a novel function to infect wild-type *E. coli* K-12 or the activation of an otherwise dormant means of entering *E. coli* K-12 present within the wild-type ΦX174 strain.

A ΦX174 mutant strain, JACS-K, created within our laboratory via extreme heat-induced mutagenesis (5a), was found to be capable of infecting both its native host *E. coli* C and the novel host *E. coli* K-12. In contrast with previous studies, no intermediary host is necessary.

In order to identify the mutations unique to the JACS-K

isolate, the phage's DNA was extracted using the UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA) and amplified using the Platinum *Taq* kit (Invitrogen, Carlsbad, CA) by 12 sets of PCR primer pairs (synthesized by Eurofins MWG Operon, Huntsville, AL), providing a minimum of 2× coverage of the genome (primers available upon request); PCR products were purified using ExoSAP-It (U.S. Biological, Swampscott, MA) and sequenced by the University of Chicago Cancer Research Center DNA Sequencing Facility. The resulting sequences were assembled using Lasergene SeqMan (DNASTAR, Inc., Madison, WI). Comparison of the JACS-K genome sequence (GenBank accession no. GU385905) to that of its ancestral strain JACS (GenBank accession no. FJ849058) revealed the presence of one nonsynonymous mutation, A100T, within the F coding region and a synonymous mutation at nucleotide position 4784. The nonsynonymous mutation occurs at a position documented as lowly conserved (15, 9).

Both the JACS-K strain and the ancestor JACS strain were plated on the native host *E. coli* C (provided by C. Burch, University of North Carolina) and *E. coli* K-12 (ATCC 25404; obtained from ATCC) using the following protocol: 100 μl of phage, 3 ml 0.5% LB agar, and 1 ml turbid bacterial culture was overlaid on a 1.7% LB agar plate. Plates were incubated overnight at 37°C. While the JACS-K strain was capable of forming plaques on both *E. coli* host plates, the ancestral JACS strain was not. The JACS-K plaques on *E. coli* K-12 were significantly smaller than the plaques observed on the *E. coli* C plates, similar to those described previously (7). Over 40 replicates were conducted to ensure reproducibility of the mutant's infectivity of *E. coli* K-12 and plaque size. The adsorption rates for both the ancestor JACS strain and the JACS-K mutant were assessed by performing an adsorption rate assay (using the methodology described previously by Bull et al. [5]) and are listed in Table 1. The adsorption rate of the JACS-K mutant by the *E. coli* K-12 host is ~40 times worse than that possible by the *E. coli* C host.

A mutant strain, ΦX174-100, was created to confer that the mutation observed was responsible for the ability of JACS-K to infect K-12. The protocol for creating this mutant is as follows. The JACS ancestor strain was digested using the restriction enzymes SspI and PshAI (New England Biolabs, Ipswich,

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TABLE 1. Adsorption rates of the ancestor JACS and JACS-K strains in both hosts

Viral strain	Adsorption rate (ml/min)	
	E. coli C	*E. coli* K-12
JACS	1.94×10^{-10}	0
JACS-K	1.91×10^{-10}	4.98×10^{-11}

MA), and a primer (synthesized by Eurofins MWG Operon, Huntsville, AL) containing ACT at the 100th codon position of F was inserted using T4 ligase (Promega, Madison, WI). *E. coli* C was used as the competent cell grown overnight in Fraser and Jerrel's glycerol media before being transferred to fresh media, a slight modification to the otherwise used Benzinger, Kleber, and Huskey protocol (2). Construction of the spheroplast followed the protocol previously described (2). Visible plaques were observed for the ΦX174-100 mutant plated on both *E. coli* C and K-12, exhibiting the number and morphology of plaques comparable to those observed for the JACS-K strain. This suggests that the change from the nonpolar alanine to the highly reactive threonine within the F gene is responsible for the expanded host range.

Referencing the structural map of ΦX174's F protein revealed that the altered amino acid is in fact at the position furthest from the viral center (17). Thus, the JACS-K mutant, in contrast with the JACS strain, has a very reactive threonine on its virion surface which can readily have its terminal hydroxyl group phosphorylated (13). In a recent study, it was found that the *E. coli* K-12 infecting coliphages HK022 and lambda are phosphorylated at one or more of their tyrosine residues (13), and the penetration of *E. coli* K-12 by these phages is dependent on the energy supplied by phosphorylation (1). To verify if indeed the phosphorylation of the new residue on the JACS-K mutant surface was responsible for the mutant's ability to infect *E. coli* K-12, analogous to the means in which HK022 and lambda infect this same host, we inhibited phosphorylation using carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). CCCP uncouples phosphorylation and prevents lambda from infecting *E. coli* K-12 (1), without interfering with plaque formation or reducing the viability of the bacterial cells (4). Figure 1 illustrates the protocol used. As a result, none of the replicate JACS-K strains produced plaques when plated with *E. coli* K-12, indicating that phosphorylation is imperative to its ability to infect this host.

The topology of the virion particle and the results of the ΦX174-F mutation suggest that converting the surrounding codons into amino acids that can be phosphorylated will result in entry into *E. coli* K-12. Three additional mutants were created, ΦX174-101, ΦX174-102, and ΦX174-100/101/102, indicating the change in amino acids at positions 101, 102, and 100, 101, and 102. All three were found to successfully infect *E. coli* C and *E. coli* K-12, although once again, pinpoint plaques were observed, with adsorption rates comparable to those of the ΦX174-100 strain. Table 2 indicates the changes incorporated, the adsorption rates, and burst sizes. The burst size observed here is comparable to that previously reported for 40 phage/infected cell (3). Figure 2 presents the average plaque size and standard error for each mutant.

The phosphorylation of the amino acid on the viral capsid appears to have a significant role in the adsorption pathway of the JACS-K isolate, as confirmed using the same experimental
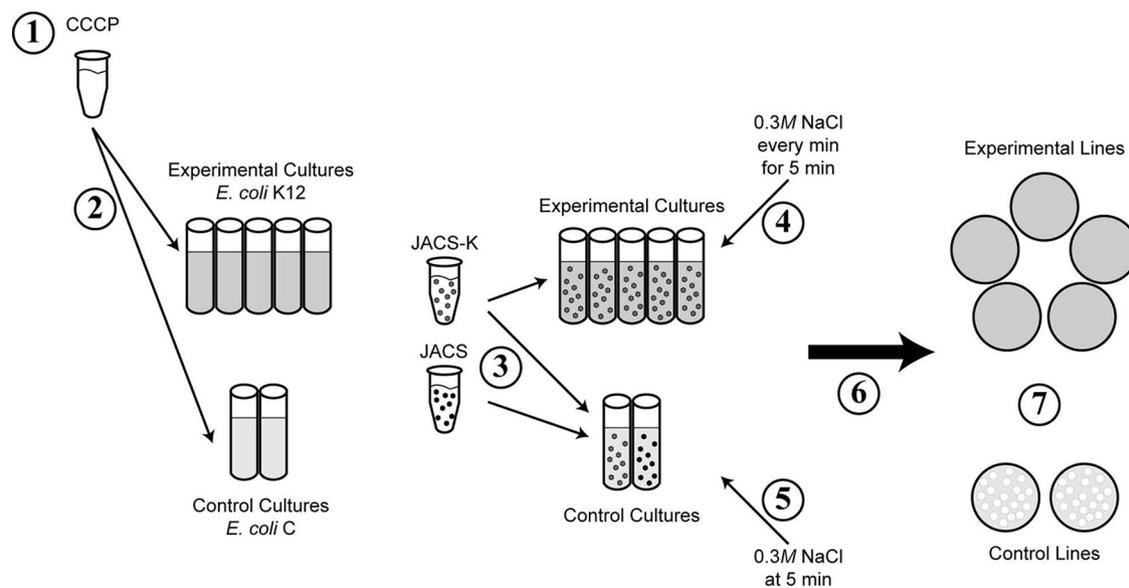

FIG. 1. Dephosphorylation experimental protocol. (1) CCCP was diluted in equal volumes of 0.01 M Tris, 0.01 M MgSO₄, and 0.01 M CaCl₂ to a final concentration of 2 M. (2) A total of 1 ml CCCP was added to five experimental liquid cultures containing 1 ml *E. coli* K-12 and to two control liquid cultures containing 1 ml *E. coli* C, and the cultures were incubated at 37°C. (3) After 5 min, 100 μl of JACS-K lysate was added to each experimental culture and one of the control tubes. A total of 100 μl of the ancestor ΦX174 JACS strain was added to the remaining control tube. (4) DNA attachment in the experimental tubes was terminated every minute for 5 min by adding cold 0.3 M NaCl. (5) DNA attachment in the control tubes was terminated after 5 min using the same method used in step 4. (6) The liquid cultures were centrifuged, and the pellet (bacteria plus any absorbed phage) was resuspended in 1 ml of saline. (7) The cultures were plated using the plating method described previously, substituting 100 μl of the suspended pellet for 100 μl of phage lysate.

TABLE 2. Phosphorylation-specific mutants and their adsorption rates and burst sizes[a]

Strain	Mutation(s)	Adsorption rate (ml/min)		Burst size (phage/infected cell)	
		E. coli C	E. coli K-12	E. coli C	E. coli K-12
ΦX174-100	A100T	1.91×10^{-10}	4.98×10^{-11}	174	35
ΦX174-101	G101S	1.83×10^{-10}	7.31×10^{-11}	170	31
ΦX174-102	Y102S	1.92×10^{-10}	5.93×10^{-11}	168	39
ΦX174-100/101/102	A100T, G101S, Y102S	1.96×10^{-10}	5.47×10^{-11}	179	44

[a] It is important to note that the amino acid in position 102 is tyrosine, which can be phosphorylated, albeit under more restrictive conditions than those for serine or threonine.

protocol employed to verify lambda's entry mechanism. While the small plaques and lower adsorption rate of the JACS-K mutant initially suggested that a single phosphorylation event is not optimal for infection to occur, the results for ΦX174-100/101/102 suggest that additional conversion sites do not increase plaque size or adsorption rate. While lambda has several tyrosine residues that are phosphorylated during the lysogenic pathway, controlling the specific timing of cell lysis (13), it benefits by encoding a protein kinase that can auto-phosphorylate as well as dephosphorylate, allowing lambda to balance the lysogenization rate with its own growth rate (13). ΦX174 does not encode a kinase, limiting its control of E. coli K-12 lysis.

The ability to be phosphorylated supports a new pathway of adsorption. Despite this, the fact that only a single mutation is necessary for the ΦX174 JACS strain to expand its host range suggests that this alternative means of entry into nonnative hosts may be exploited in nature, analogous to observations made within Φ6 (8). Within bacteria, it has been observed that the alteration of just one or two amino acids into a phosphoprotein can dramatically alter the protein's function, a mechanism frequently employed (12). Interestingly, the threonine at amino acid position 100 in the F protein is ubiquitous among the 66 complete ΦX174 genomic sequences available in GenBank. Moreover, the presence of Ser101 or Ser102 appears in five publicly available genomes (GenBank accession no. AF274751, M14428, DQ079892, EF380013, and EF380025). As the majority of ΦX174 experiments in the literature examine the phage's infection of bacterial

strains in which the LPS is present on the host's outer membrane, the ability to infect K-12 has been largely ignored.

The JACS-K isolate was propagated in triplicate for 21 days through liquid culture with naïve E. coli C only, E. coli K-12 only, and alternating hosts in an effort to explore the selection upon the strain given the different host systems. While no difference in phenotype was observed, all three lineages maintained their ability to infect K-12. Increased fitness, at least with respect to traits preferable within the laboratory, like plaque size, may require more than a means of entry to infect E. coli K-12 at a rate comparable to those of its native hosts. We hypothesize that the acquisition of the same mutation observed here (or one conveying the ability to phosphorylate) occurred in the study of Bone and Dowell (3) during the phage's replication within the intermediary mutant E. coli K-12 host.

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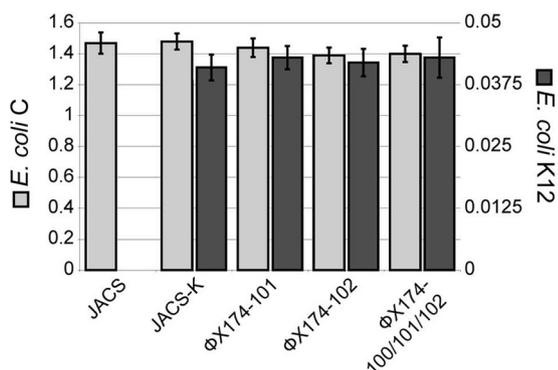14. Reference deleted.

FIG. 2. Average plaque size (mm). Phage plated on *E. coli* C are shown with the light gray bars, with sizes corresponding to the axis on the left; phage plated on *E. coli* K-12 are shown with the dark gray bars, with sizes corresponding to the axis on the right. Standard errors of the means are shown.

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