Isolation and Partial Characterization of Sex-specific Bacteriophages Infective for Escherichia coli K12[1]

JOHN H. LANCASTER, WILLIAM L. FESLER[2], and
JEANITA W. BELFORD, University of Oklahoma, Norman

The occurrence of sex-specific bacteriophages was first reported by Loeb (1960), who isolated seven phages showing a specificity for male *Escherichia coli* mating types. Six of these isolates were small, spherical phages containing RNA. The seventh was a filamentous, single-strand DNA phage (Zinder, et al., 1963). Since Loeb's original observations, a number of workers have reported isolation of male-specific phages (Dettori, Maccacaro and Puccinin, 1961; Marvin and Hoffman-Berling, 1963; Bradley, 1964). All of these isolates have been absolutely male-specific. They do not adsorb to female cells.

In addition to a number of male-specific phages, Dettori, Maccacaro and Puccinin (1961) also isolated eight female-specific phages. These phages were not absolutely specific. They will infect male cells at approximately one-thousandth the efficiency with which they will infect female mating types.

This communication reports the isolation and partial characterization of a number of male- and female-specific phages isolated in our laboratory.

Materials and Methods

Organisms—The organisms used in this study were:

Escherichia coli K12 Hfr G5 *his*$^-$; *E. coli* K12 F$^-$464 *ileu*$^-$; and bacteriophage MS-2. The bacteria were maintained routinely on nutrient agar. Bacteriophage MS-2, a male-specific, spherical RNA phage, was maintained as culture lysates in nutrient broth. Both bacterial strains and phage MS-2 were kindly provided by Dr. E. P. Goldschmidt, Biology Department, University of Houston.

Bacteriophage isolation—Raw sewage collected from the municipal sewage disposal plant at Norman, Oklahoma, was used as a source of phage. Plaques obtained on soft agar overlays (Adams, 1959), seeded with donor (Hfr G5) or recipient (F-464) cells, were transferred to log-phase, broth cultures of the same mating type used to seed the overlay. These infected cells were incubated at 37 C with agitation in a New Brunswick Gyrotory shaker for 6 hr, or until the cultures lysed. Unlysed cells and cell debris were removed by centrifugation at 3000 $\times$ g and the phages remaining in the supernate were screened for sex specificity by cross-streaking against Hfr G5 and F$^-$464 cells. Those phages which showed indications of sex specificity were tested against the two mating types by broth culture lysis and by the agar overlay technique.

Preparation of phage antisera—Antisera were prepared in rabbits

[1]This work was supported by NIH Grant AI-07221.
[2]Current address: University of Oklahoma School of Medicine, Oklahoma City.

against bacteriophages MS-2 and FSO-1, a female-specific isolate. Intraperitoneal injections were performed weekly over a two-month period. Each injection consisted of 4 ml of a phage suspension containing 1×10^9 PFU per ml. All phage isolates were tested against both antisera, using the infectivity neutralization assay described in Adams (1959).

Latent period determination—The latent period of a phage infection is that time interval extending from the initial infection of host cells to the first appearance of virus particles released by natural lysis of these cells. The extent of the latent period was measured using the one-step growth technique described in Adams (1959).

RESULTS

Sex-specificity of bacteriophage isolates—The sex specificity of representatives of each serological group is shown in Table I. MSO-7, MSO-11 and FSO-1 are highly sex-specific in their host range. These phages produced no plaques on the nonsensitive hosts when 0.1 ml of stock phage suspension was used as inoculum in soft-agar overlay assays. Phages with an MSO designation are male-specific. Those of the FSO series are female-specific. MSO-12 does not show absolute male specificity. This is apparently due to the presence of a nonspecific contaminating phage which we have so far been unable to separate from MSO-12, even after repeated single-plaque isolations. A similar condition exists in MSO-9, a member of serological group III.

FSO-12 is the only member of the female-specific phage series to demonstrate infectivity for male cells. In this respect it is similar to the female-specific phage described by Dettori, Maccacaro and Puccinin (1961).

Serological and latent time characterization of phage isolates—Table II summarizes the data obtained from infectivity neutralization tests performed with MS-2 and FSO-1 antisera. Group I, representing nine male-specific isolates, is reactive with neither MS-2 nor FSO-1 antisera. Group II, consisting of MSO-11 and MSO-9, is slightly inactivated by both antisera. Group III, consisting of MSO-12 and MSO-14, is strongly reactive with MS-2 antiserum, but is nonreactive with anti-FSO-1 serum. These isolates seem to be serologically identical with MS-2.

All of the female-specific isolates are identical with FSO-1, except for FSO-12. This phage is nonreactive with MS-2 antisera and only slightly reactive with antiserum against FSO-1.

These serological groups, especially those involving male-specific phage, represent a minimum number of divisions. Group I and Group II may both consist of multiple serological types whose specificity could not be detected with the two antisera used. Even in Group III, whose serological relation to MS-2 is apparently very close, we see a significant variation in latent times between MSO-12 and MS-2.

DISCUSSION

Most of the reported work involving sex-specific phages has centered around those that are infectious for male *Escherichia coli* mating types. There are two reasons for this. First, most of the male-specific phages are small, spherical RNA phages. These phages have proved to be extremely valuable as tools for study of RNA virus replication and the properties of RNA as genetic material (Zinder, 1965). The male-specific DNA phages are also unique in their filamentous morphology, their mode of liberation from infected host cells, and their single-strand DNA (Hofschneider and Preuss, 1963; Zinder et al, 1963)

Secondly, attachment of male-specific phage to susceptible hosts is dependent on the presence of structures on the cell surface whose synthe-

sis is under the genetic control of fertility factors, which confer maleness on *E. coli* cells. In all cases so far studied, these phage receptor sites have been F-pili (Brinton, Gemski and Carnahan, 1964). Surface factors which have properties different from those of F-pili also appear to be involved in conjugation (Lancaster, Goldschmidt and Wyss, 1965; Lancaster, unpublished results). Similar substances have also been obtained from female cells (Lancaster, Goldschmidt and Wyss, 1965; Schwartz, Eiler and Kern, 1965).

We undertook the isolation of these sex-specific phages with the object of using them as tools for the study of these sex-related surface characteristics. Specifically, we wish to determine if the phage receptor sites are identical to the surface factors involved in conjugation, and how these surface factors are genetically controlled.

We have not been able to demonstrate adsorbence of our male-specific phage isolates to F-pili, but our techniques of assay for adsorbence have not been sufficiently refined to consider these negative results as significant.

The fact that our female-specific phage of the FSO-1 group appears to be highly, if not absolutely, specific for female cells is of great interest from the standpoint of studies concerning sex-related surface characteristics. Mating type in *E. coli* male cells may be phenotypically altered by cultivating the cells under conditions which will apparently alter the

TABLE I. SEX SPECIFICITY OF BACTERIOPHAGE ISOLATES

Bacteriophage Isolate	Titer on F-464 (PFU / ml.)	Titer on Hfr G6 (PFU / ml.)	Serological Group
MSO-7	<10	1.6×10^{9}	I
MSO-11	<10	4.0×10^{9}	II
MSO-12	6.0×10^{3}	3.0×10^{8}	III
FSO-1	7.0×10^{8}	<10	IV
FSO-12	2.0×10^{8}	5.0×10^{5}	V

TABLE II. SEROLOGICAL GROUPING AND LATENT PERIOD DETERMINATION OF SEX-SPECIFIC BACTERIOPHAGE ISOLATES

Bacteriophage Isolate	Neutralization by MS-2 Antisera	Neutralization by FSO-1 Antisera	Serological Group*	Latent Time
MSO-7	—	——	I(9)	25 min.
MSO-11	+	+	II(2)	20 min.
MSO-12	++++	——	III(3)	8 min.
FSO-1	—	++++	IV(18)	20 min.
FSO-12	—	+	V(1)	8 min.
MS-2	++++	——	III	14 min.

* The numbers in parentheses represent the total number of isolates found in each serological group.

expression of the fertility factor (Jacob and Wollman, 1961). Under these conditions (i.e., cultivation under heavy aeration) male cells will behave phenotypically as females. Three hypotheses may be offered as explanation for this observation: (1) All *E. coli* cells are fundamentally female and the presence of the fertility factor results in the production of some substance associated with maleness which coats and masks the female surface substance; (2) the presence of the fertility factor inhibits the expression of chromosomal genes responsible for femaleness, and under cultural conditions which prevent expression of maleness, this inhibition is relieved and femaleness is expressed; or (3) genetic expression of femaleness is not completely blocked by the activity of the fertility factors. Instead, an altered female material may be produced which some phages (i.e., FSO-12) can use as receptor sites while others, such as FSO-1, cannot.

The host range of phages such as FSO-12 could be explained by the first hypothesis if we assume an incomplete coating of female material by the male substance, leaving some female-specific phage receptor site exposed. This would not, however, account for the high level of female specificity shown by phages such as FSO-1. Conversely, the host range specificity of FSO-1 is compatible with hypothesis (2), while that of FSO-12 is not. The third hypothesis is the only one of the three which can explain the host range of both phages FSO-1 and FSO-12. Experiments are currently under way to test this hypothesis by isolating and characterizing female-specific phage receptor sites from female cells, male cells and male cells of female phenotype.

SUMMARY

Fourteen male-specific and 19 female-specific bacteriophages were isolated from sewage. These were subdivided into five serological groups on the basis of their cross-reactivity with antisera against one female-specific phage (FSO-1) and one male-specific phage (MS-2). Representatives from each serological group were tested for variations in the length of their latent periods, which were significantly different between groups. All the phage isolates were tested for their infectivity on male and female hosts. All male-specific phages appear to be infectious only for male cells. Eighteen of the female-specific isolates do not infect male cells. All of these belong to the same serological group. The remaining female-specific phage has a reduced, but significant, infectivity for male cells. A tentative hypothesis explaining the differences in host-range among the female-specific isolates is offered.

LITERATURE CITED

Adams, Mark H. 1959. *Bacteriophages*. Interscience Publishers, Inc. New York.

Bradley, D. E. 1964. Some preliminary observations on filamentous and RNA bacteriophages. J. Ultrastruct. Res. 10:385-389.

Brinton, C. C., P. Gemski and J. Carnahan. 1964. A new type of bacterial pilus genetically controlled by the fertility factor of *Escherichia coli* K12 and its role in chromosome transfer. Proc. Nat. Acad. Sci. 52:776-783.

Dettori, R., G. A. Maccacaro and G. L. Puccinin. 1961. Sex-specific bacteriophages of *Escherichia coli* K12. Microbiol. 9:141-150.

Hofschneider, P. H. and A. Preuss. 1963. M13 bacteriophage liberation from intact bacteria as revealed by electron microscopy. J. Mol. Biol. 7:450-451.

Jacob, F. and E. Wollman. 1961. *Sexuality and the Genetics of Bacteria*. Academic Press, New York.

Lancaster, J. H., E. P. Goldschmidt and O. Wyss. 1965. Characterization of conjugation factors in *Escherichia coli* cell walls. I. Inhibition of recombination by cell walls and cell extracts. J. Bacteriol. 99:1478-1481.

Loeb, T. 1960. Isolation of a bacteriophage specific for the F+ and Hfr mating types of *Escherichia* coli K12. Science 131:932-933.

Marvin, D. and H. Hoffman-Berling. 1963. Physical and chemical properties of two new small bacteriophages. Nature 197:517.

Schwartz, G. H., D. Eiler and M. Kern. 1965. Solubilization of the conjugation inhibitor from *Escherichia coli* cell wall. J. Bacteriol. 89:89-94.

Zinder, N. D. 1965. RNA phages. Ann. Rev. Microbiol. 19:455-477.

Zinder, N. D., R. E. Valentine, M. Roger and W. Stockenius. 1963. F1, a rod-shaped male-specific bacteriophage that contains DNA. Virol. 20:638-642.