Loss of the F factor in Escherichia coli in motility medium
by Cheng Wou Yu

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Abstract:
A phenomenon observed in E.coli K-12 is that one can readily isolate an F- cell from the edge of an F+
population in a swarm. The present study attempts to assess the role of various factors possibly
responsible for this phenomenon.

In these experiments a method to visualize the formation of F-populations within motile swarms of F+
cultures in motility medium was developed. The new technique employed the following two aspects:
(1) Lactose and 2,3,5-triphenyl tetrazolium chloride as an indicator were added to the motility medium;
thus any zone of Lac- cells will be red while Lac cells will be white. (2) A recA-lac-/F'-lac+
merodiploid (F42/JC1553) was used in which the lac+ gene marked the fertility factor, and the former
was prevented from crossing over to the bacterial chromosome by the presence of the recA~ gene; thus
any Lac- phenotypes which arise in a Lac+ strain will represent the loss of the fertility factor.

In this strain, the rate of F factor reinfection in unagitated liquid culture is estimated to be 0.08 percent
where the cell concentration is 5x10/ml for both F+ and F- cells. The cell density at the area just ahead
of the visible edge of a swarm is found to be less than 5x104/ml; this is the generative part of the
swarm giving rise to the majority of cells in subsequent enlargements of the swarm. Furthermore, the
movement of cells in the swarm is highly non-random; that is, related cells tend to be associated in the
same sector. The low cell concentration experienced by ancestors of swarm edge cells together with the
geographical isolation of clones within a swarm automatically minimizes the probability of reinfection
of newly arisen F- clones.

During the formation of a swarm most initial "flares" that extend beyond the major swarm area contain
only F- cells; thus either selection of F- cells per se or enhanced loss of F factor in fast cells or both are
involved. Since selection (faster swarming of F- cells) has been demonstrated elsewhere, this is
probably the important factor here.

The basic rate of spontaneous loss of the fertility factor at 37°C in a liquid medium is about 3x10-3
losses per cell division. The high rate of spontaneous loss of the F factor, the selective advantage of F~
cells, and the minimization of reinfection in swarms seem to be the major factors responsible for the
ease with which F- cells can be isolated from F+ swarms.

LOSS OF THE F FACTOR BY <u>ESCHERICHIA</u> <u>COLI</u> IN
MOTILITY MEDIUM

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ABSTRACT

A phenomenon observed in $\underline{E}$. $\underline{coli}$ K-12 is that one can readily isolate an F^- cell from the edge of an F^+ population in a swarm. The present study attempts to assess the role of various factors possibly responsible for this phenomenon.

In these experiments a method to visualize the formation of F^- populations within motile swarms of F^+ cultures in motility medium was developed. The new technique employed the following two aspects: (1) Lactose and 2,3,5-triphenyl tetrazolium chloride as an indicator were added to the motility medium; thus any zone of Lac^- cells will be red while Lac^+ cells will be white. (2) A $\underline{recA}^-\underline{lac}^-/F'-\underline{lac}^+$ merodiploid (F42/JC1553) was used in which the $\underline{lac}^+$ gene marked the fertility factor, and the former was prevented from crossing over to the bacterial chromosome by the presence of the $\underline{recA}^-$ gene; thus any Lac^- phenotypes which arise in a Lac^+ strain will represent the loss of the fertility factor.

In this strain, the rate of F factor reinfection in unagitated liquid culture is estimated to be 0.08 percent where the cell concentration is 5×10^4/ml for both F^+ and F^- cells. The cell density at the area just ahead of the visible edge of a swarm is found to be less than 5×10^4/ml; this is the generative part of the swarm giving rise to the majority of cells in subsequent enlargements of the swarm. Furthermore, the movement of cells in the swarm is highly non-random; that is, related cells tend to be associated in the same sector. The low cell concentration experienced by ancestors of swarm edge cells together with the geographical isolation of clones within a swarm automatically minimizes the probability of reinfection of newly arisen F^- clones.

During the formation of a swarm most initial "flares" that extend beyond the major swarm area contain only F^- cells; thus either selection of F^- cells $\underline{per}$ $\underline{se}$ or enhanced loss of F factor in fast cells or both are involved. Since selection (faster swarming of F^- cells) has been demonstrated elsewhere, this is probably the important factor here.

The basic rate of spontaneous loss of the fertility factor at 37°C in a liquid medium is about 3×10^{-3} losses per cell division. The high rate of spontaneous loss of the F factor, the selective advantage of F^- cells, and the minimization of reinfection in swarms seem to be the major factors responsible for the ease with which F^- cells can be isolated from F^+ swarms.

INTRODUCTION

The sexuality of bacteria was not clear until 1946, when Lederberg
and Tatum showed that mutants of _Escherichia coli_ K-12 could recombine
genetically with each other. During the early 1950's, the determina-
tion of bacterial fertility was under study in several laboratories,
especially by Lederberg in Wisconsin and by Hayes in London. It was
discovered that certain ("male") strains of _E. coli_ contain an extra-
chromosomal genetic element known as a sex factor, and that a cell of
this type is capable of transferring its genetic material to a recip-
ient "female" cell, which lacks a sex factor (Lederberg, Cavalli and
Lederberg, 1952; Hayes, 1953; Cavalli, Lederberg and Lederberg, 1953).
The male determining factor is called the fertility factor, or F factor,
or in shart, F. Strains, containing this fertility factor are referred
to as F^+, those without as F^- (Lederberg, Cavalli and Lederberg, 1952;
Hayes, 1953). The F factor is a double helix DNA molecule with a
molecular weight of 63×10^7 daltons (Low, 1972). A fertility factor can
replicate autonomously in a host bacterium, or it can integrate into
the bacterial "chromosome". In the latter case, the male cell is call-
ed Hfr (Cavalli-Sforza, 1950). Sometimes chromosomal genes come to be
associated with the autonomous sex factor by a reciprocal recombination
process; in such cases the F factor is referred to as F prime, or F'
(Jacob and Adelberg, 1959).

Several strains of _E. coli_ including K-12 are motile by virtue
of several flagella distributed around the cell. When a motile strain

of E. coli is inoculated in a semisolid agar (motility medium), some
of the bacteria swim away from the site of inoculation while the others
remain in the center.

Transferring an F^+ strain in motility medium and selecting from
the edge of swarm enable one to isolate an F^- strain from an F^+ popu-
lation. Skaar et al, (1957) concluded that F^- cells swim faster than
F^+ cells and that this was a sufficient explanation for the above
phenomenon. The apparently complete stability of the swarm derived F^-
cells together with the fact that they can readily be reconverted to
F^+ cells argue that these cells are neither phenocopies nor defective
F^+ but have indeed lost their fertility factors.

It has been known for a long time that the sex factor (or F') in
an F^+ strain can be lost (Jacob and Wollman, 1961). To use the F prime
lactose (F'-lac^+) as an example, lac^-/F'-lac^+ cells will segregate
bacteria which have the Lac⁻ phenotype at a rate of about 10^{-3} segrega-
tions per cell division (Jacob and Adelberg, 1959). However, these
segregants include both F^- cells and homogenotes lac^-/F'-lac^-.

F^- cells can be obtained with high frequency from F^+ strains fol-
lowing treatment with different materials (a curing effect) such as
acridine dyes (Hirota, 1960; Bastarrachea and Willetts, 1968), rifampin
(Bazzicalupo and Tocchini-Valentini, 1972; Riva et al, 1972), thymine
deprivation (Clowes et al, 1965), filamentous phage M13 (Palchoudhury
and Iyer, 1971), or following growth at 43°C (Stadler and Adelberg,

1972). The mechanisms underlying spontaneous loss and curing are still not understood.

Most attention in the past has been focused on the curing phenomenon. Few studies on spontaneous loss, especially in motility medium, have been done. The present studies are aimed at testing the "spontaneity" of fertility factor loss on motility medium and at defining the factors that are responsible for the ease with which F^- cells can be isolated on this medium.

MATERIALS AND METHODS

Bacterial Strains

The bacterial strains employed are listed in Table 1. All are derived from E. coli K-12. Strains AB2463, NH4104, F42/JC1553 and JC1553 were obtained from E. coli stock center, Yale University. MSU2000, MSU2001, and MSU2002 were isolated by the author.

Media and Supplements

Final concentrations are given as grams/liter of distilled water unless otherwise indicated. Media were sterilized in an autoclave (AMSCO) at 24 pounds pressure for 20 minutes before use.

Nutrient agar: Difco Nutrient Broth 8.0, NaCl 5.0, agar 15.0.

Penassay Broth (PB): Bacto-Beef Extract 1-5.0, Bacto-Yeast Extract 1.5, Bacto-Peptone 5.0, Bacto-Dextose 1.0, NaCl 3.5, K_2HPO_4 3.6, KH_2PO_4 1.3.

Eosin Methylene Blue Agar (EMB): Bacto-Casitone 8.0, Bacto-Yeast Extract 1.0, NaCl 5.0, K_2HPO_4 2.0, Eosin Y 0.6, Methylene Blue 0.065, sugar 10.0, agar 15.0.

Motility Medium: Bacto-Peptone 10.0, Bacto-Yeast Extract 3.0, Bacto-Gelatin 8.0, NaCl 5.0, agar 4.0.

Motility (TZ) Medium: Bacto-Peptone 10.0, Bacto-Yeast Extract 3.0, Bacto-Gelatin 80.0, NaCl 5.0, agar 4.0, lactose 10.0, 2,3,5-triphenyl tetrazolium chloride 50 mg. The carbon source is added after autoclaving.

Table 1. Bacterial strains.

Strain	Malting type	lac Genotype	Auxotrophic markers	Other markers and properties
W1895	HfrC	$\underline{lac}^+$	$\underline{met}^-$	$\underline{rec}^+$, $\underline{str}^s$
MSU2000	HfrC	$\underline{lac}^+$	$\underline{met}^-$	$\underline{rec}^+$, $\underline{str}^s$, fast swarming
AB266	F^-	$\underline{lac}^-$	$\underline{thr}^-$, $\underline{leu}^-$, $\underline{thi}^-$, $\underline{pro}^-$	$\underline{rec}^+$, $\underline{str}^r$
MSU2001	F^+	$\underline{lac}^-/\underline{lac}^+$	$\underline{thr}^-$, $\underline{leu}^-$, $\underline{thi}^-$, $\underline{pro}^-$	$\underline{rec}^+$, $\underline{str}^r$, F42 episome
AB2463	F^-	$\underline{lac}^-$	$\underline{thr}^-$, $\underline{leu}^-$, $\underline{thi}^-$, $\underline{arg}^-$, $\underline{his}^-$, $\underline{pro}^-$	$\underline{recA13}$, $\underline{str}^r$
NH4104	F^+	$\underline{lac}^-/\underline{lac}^+$	$\underline{thr}^-$, $\underline{leu}^-$, $\underline{thi}^-$, $\underline{his}^-$, $\underline{pro}^-$	$\underline{rec}^+$, $\underline{str}^s$, F42 episome
F42/JC1553	F^+	$\underline{lac}^-/\underline{lac}^+$	$\underline{arg}^-$, $\underline{met}^-$, $\underline{his}^-$, $\underline{leu}^-$, $\underline{pro}^-$	$\underline{recA1}$, $\underline{str}^r$, F42 episome
MSU2002	F^-	$\underline{lac}^-$	$\underline{arg}^-$, $\underline{met}^-$, $\underline{his}^-$, $\underline{leu}^-$, $\underline{pro}^-$	$\underline{recA1}$, $\underline{str}^r$, fast swarming
JC1553	F^-	$\underline{lac}^-$	$\underline{arg}^-$, $\underline{met}^-$, $\underline{his}^-$, $\underline{leu}^-$	$\underline{recA1}$, $\underline{str}^r$

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Tetrazolium Indicator Plates (TZ): Methods for preparing this medium are described by Ohlsson et al., (1968). Bacto-Beef Extract 1.5, Bacto-Yeast Extract 3.0, Bacto-Peptone 6.0, agar 15.0, 2,3,5-triphenyl tetrazolium chloride 50 mg, Lactose 10.0, 2,3,5-triphenyl tetrazolium chloride heating before autoclaving. After autoclaving, the carbon source is added to a final concentration of 1%. On this medium, colonies which utilize the sugar are white, those which do not are red, and strains suppressed for sugar utilization are intermediate shades of pink.

M medium: Bacto-Peptone 10.0, Bacto-Yeast Extract 3.0, NaCl 5.0.

M medium (TZ): 2,3,5-triphenyl tetrazolium chloride 50 mg, Lactose 10.0 are added to M medium.

Saline: NaCl 8.6.

Streptomycin: Dihydro-streptomycin sulfate 250 mg/ml.

Methods (Unless stated otherwise cultures were incubated at 37°C)

(a) Transferring in motility medium and isolation of an F⁻.

An overnight PB broth culture of F42/JC1553 was diluted to a concentration of 10^7 cells/ml. A loopful of bacteria was deposited in the center of a motility medium which was then incubated for 18 to 24 hours with plate face up. The next transfer was made by placing a loopful from the edge of the swarm of the first day in 1 ml of saline. A loopful of this diluted culture was then immediately transferred to a new plate. Simultaneously, a loop of diluted culture was further

diluted and its cell types are assayed by plating on tetrazolium plates. Lac$^+$ (or F$^+$) and Lac$^-$ (or F$^-$) colonies were scored by the different colors of the colonies after 18 to 20 hours of incubation. This method was repeated for several days until a completely F$^-$ swarm was isolated. Bacteria from the edge of a swarm were streaked on tetrazolium indicator plate and an insolated colony was selected. This isolated colony was checked for its chemical markers and a slant was made.

(b) Measurement of the swarming rate.

F42/JC1553 was transferred in motility medium as described in (a) and each transfer is referred to a passage. After incubation of each transfer, the radius of the swarm was measured by a ruler in terms of mm at the time of making a new transfer. Swarming rate was then calculated by dividing the radius by the age of the swarm (time of incubation).

(c) Calculation of reinfection

The method is similar to that described by Hayes (1968). A log phase broth culture of an Lac$^+$ F$^+$ strain (F'-lac$^+$) and a Lac$^-$ F$^-$ strain of equal population densities and dilutions of this culture were mixed in equal amounts. The former carries a streptomycin sensitive gene and the latter resistance. At various times samples were removed from the mixture, diluted and plated on EMB lactose agar plates which had been spread with 0.1 ml of streptomycin. Plates were then incubated for 16 to 18 hours and Lac$^+$ and Lac$^-$ colonies were counted. All Lac$^+$

phenotype colonies were attributable to a transferring of the F prime
(F'-$\underline{lac}^+$) from the donor strain ($\underline{str}^s$) to the recipient ($\underline{str}^r$). A
control plate was made to prove the killing effect of streptomycin on
the donor strain.

(d) <u>Measurement of the rate of spontaneous loss of the fertility</u>

<u>factor</u>

<u>Median culture method</u>: Overnight PB broth culture of
F42/JC1553, an F^+ Lac^+ $RecA^-$ strain with genotype $\underline{lac^-recA^-}/F'$-$\underline{lac}^+$,
was diluted to concentration of 10^2 cells/ml and a sample of 0.1 ml
of the diluted culture was transferred to 9.9 ml of M medium for
further growth. The M medium was incubated in a water bath till the
estimated population density in the tubes exceeded 10^4/ml but was not
more than 4×10^4/ml. All the tubes were removed and immediately dipped
into crushed ice to stop cell division. A 1 ml sample was taken from
each tube and 0.1 ml was plated on 10 tetrazolium indicator plates
which were then incubated for 18 to 20 hours. The total number of
colonies and Lac$^-$ phenotypes (with red color) on each plate were counted.
From the median number of mutants a mean number of mutants per culture
can be calculated from the chart published by Lea and Coulson (1949).
From this, an estimated mutation rate was then obtained.

<u>Negative culture method</u>: An overnight culture of F42/JC1553 was
diluted to a concentration of 10^2 - 5×10^2 cells/ml. The bacteria were
inoculated into either 10 plates or 10 tubes. In the "plate method",

0.2 ml of bacteria was spotted on the center of a tetrazolium plate

(TZ) and incubated for 2.5 to 3 hours with plates face up and then

spread at the end of incubation. Plates were then reincubated for 18

hours. The number of plates on which Lac$^-$ phenotype did not occur was

scored. In the "tube method", 0.1 ml of diluted culture was inoculated

into a tube with 0.9 ml of M medium. Tubes were incubated for 2.5 to

3 hours. At the end of incubation period the tube was poured onto a

TZ plate and spread. The empty tube was washed with 0.5 ml of saline,

then poured on other TZ plate and spread. This was repeated once more

to wash the bacteria out of the tube more completely. The tetrazolium

plates were incubated for 18 to 20 hours. The fraction of plates (or

tubes) which did not contain any Lac$^-$ cell(s) (with a red color accu-

mulated in the colony) was scored; this fraction is P_o. From the

Poisson term $P_o = e^{-m}$, the mean number of mutations per culture can

be calculated. Division of m by the total number of cell divisions

that have occurred in the culture gives an estimated mutation rate.

RESULTS

Background studies of strain F42/JC1553

In order to facilitate studies of the loss of F factor on motility

medium, the strain F42/JC1553 was used. This strain is a lac^-/lac^+

heterozygous merodiploid in which the lac^+ allele is associated with

the F factor. The genotype for this heterogenote is $recA^- lac^-/F'-lac^+$.

This strain is also RecA, thus obviating either any confusion of true

F factor loss with crossing over leading to a $lac^-/F'-lac^-$ homozygous

merodiploid, or any undetected loss of the F factor which occurred after

a reciprocal recombination involving the lac^- and lac^+ alleles resulting

in a lac^+ haploid. Any loss of the $F'-lac^+$ will retard this bacterium's

ability to utilize lactose as well as the ability to convert an F^-

strain to an F^+.

Since the early work on derivation of F^- cells on motility medium

employed Rec^+ cells carrying a wild type F factor, it was necessary

first to verify certain properties of F42/JC1553.

The behavior of F42/JC1553 on motility medium

Swarming rate. Table 2 illustrates the increase in swarming rate

exhibited by six genetically different strains during repeated passage

on motility medium (the method of calculating swarming rate is justified

by the generally linear growth of swarm radii shown in Figure 1). All

strains exhibit a progessive increase in swarming rate, but it will be

noticed that the $RecA^-$ strains are less responsive. This could be a

mere coincidence, since no two strains are isogenic except for the $recA$

Table 2. Swarming rate (mm/hr) of six strain.

Passage			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age of swarm at time of measurement (hrs)			23	22	9	15	10	15	18	17	10	27	22	21	21	21	21

Strain	sex	rec															
W1895	Hrf	+	1.2	1.7	2.1	1.9	2.0	2.1	1.9	2.6	3.6	–	–	–	–	–	–
AB266	F⁻	+	1.1	1.5	1.7	1.6	1.6	1.8	1.8	2.3	3.3	–	–	–	–	–	–
AB2463	F⁻	–	0.7	0.7	0.7	0.7	0.6	0.9	0.8	0.8	0.8	–	–	–	–	–	–
NH4104	F⁺	+	1.2	1.7	2.0	1.9	1.7	1.9	1.8	2.4	3.2	–	–	–	–	–	–
F42/JC1553	F⁺	–	0.5	0.7	0.6	0.9	0.7	0.8	0.9	1.2	0.8	1.3	1.3	1.5	1.6	1.7	1.7
JC1553	F⁻	–	0.6	1.0	1.1	1.3	0.8	0.9	1.1	1.2	0.9	1.3	1.4	1.5	1.6	1.7	1.7

The inoculum for the initial passage was 1 loopful (about 0.025 ml) of an overnight PB culture. Subsequent inoculations were single loopful picked from the visible edge of the swarm from the preceding passage at the time of its measurement. Rates given were derived by dividing the radius of the swarm (in mm) by its age (in hours).

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locus, or it could be a reflection of different growth rates. On the other hand, it could mean that one component of enhanced motility is a phase variation shift under recA control (Lederberg, 1973). It will also be noticed that strain JC1553, isogenic with F42/JC1553 but lacking the F' factor, is in this experiment slightly faster than F42/JC1553 in the first few transfers. Although the repeatability of this difference has not been tested, it is consistent with the findings of this difference has not been tested, it is consistent with the findings of Skaar et al (1957) on other isogenic F^+ and F^- strains. Furthermore, an interesting finding is that the initial cell concentration of inoculation is also a factor which influences the swarming in terms both of the time swarming starts and eventual swarming rate (Fig. 1).

Isolation of F^- cells from swarms. Despite the fact that F42/ JC1553 never swarmed as fast as the Rec^+ strains tested, it was relatively easy to isolate Lac^- (or F^-) cells by streaking from the edge of swarm after they had shown some degree of motility enhancement, particularly if one selected from the fast "flares" that appear on the early passages.

Rate of reinfection in liquid medium

F^+ cells rapidly convert F^- cells to F^+ when the two are mixed in high densities. Such reinfection is an obvious factor to be considered in understanding the origin of F^- from F^+ cells on motility medium. Therefore the efficiency with which NH4104 (F^+) converts the F^- strains AB266 and MSU2002 was measured at various concentrations and at various
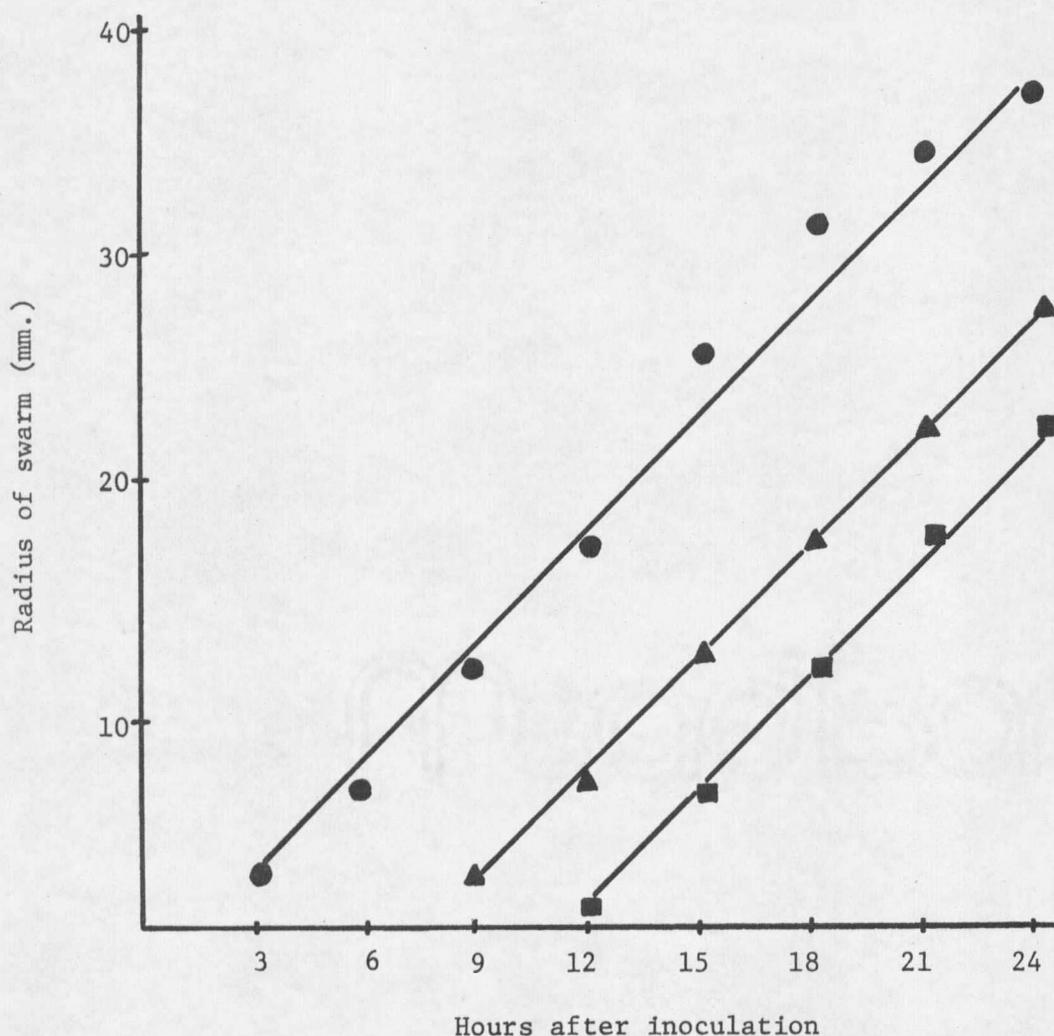
Figure 1. Swarming rate of F42/JC1553 in motility medium as a function
of inoculum size. A loopful (about 0.025 ml) of F42/JC1553
was transferred to motility medium and the radius of the
swarm was measured at 3 hour intervals. The circles repre-
sent measurements of a swarm derived directly from an over-
night PB culture (inoculum = ca. 2.5×10^6/ml). The trian-
gles represent a swarm derived from a similar inoculum
diluted about 1/400. The squares represent a swarm derived
from an inoculum diluted about 1/4000.

times of joint incubation in PB and M medium. The results shown in
Table 3 reveal no marked difference in the efficiencies with which
AB266 and MSU2002 are reinfected, nor in the efficiency of reinfection
in PB (a common medium for earlier experiments) and M medium (the
liquid equivalent of motility medium). Furthermore, although NH4104
carries the episome F42 (as does F42/JC1553) the rates of reinfection
are not markedly different from those described for wildtype F. It
will be noted, incidentally, that the reinfection process does not seem
to follow simple second order kinetics. Specifically, the conversion
event is disproportionately high at low cell concentrations. The reason
for this is not clear.

Rate of loss of the F factor in liquid medium

Character of segregated colonies. Streakings of overnight PB
cultures of F42/JC1553 on tetrazolium plates usually contain some Lac^-
segregant colonies. These segregant colonies are stably F^- and are
readily reconverted to F^+ when grown with F^+ cells. This indicates
that they have indeed lost their fertility factors (Maas, 1963; Echols,
1963; Scaife and Gross, 1963). Similar streaking of a Rec^+ F^+ strain
MSU2001 ($lac^-/F'-lac^+$), show a much higher rate of segregation. In
a Rec^+ strain it is possible to obtain a Lac^- phenotype from a $lac^-/$
$F'-lac^+$ heterogenote in the following three ways: (1) simple loss of
the F factor. (2) reciprocal recombination between F' factor and
bacterial chromosome involving an insertion of the F factor and loss

Table 3. Frequencies of reinfected F⁻ cells at various cell concentrations and after various times of joint incubation.

| Conc. of each component at mixing (Cells/ml) | (A) (Time of joint incubation) | | | | | | | | | | | | (B) (Time of joint incubation) | | | (C) | | |
| | 40 min | | | 70 min | | | 100 min | | | 180 min | | | 40 min | | | 40 min | | |
	Lac+	Total	%	Lac+	Total	%	Lac+	Total	%	Lac+	Total	%	Lac+	Total	%	Lac+	Total	%
10^8	44	690	6.4	324	1821	17.8	311	1160	26.8	599	1549	38.7	136	1382	9.8	95	1163	8.17
10^7	34	839	4.1	54	933	5.8	94	864	10.9	506	1303	38.8	20	817	2.5	19	1304	1.45
10^6	10	600	1.7	22	561	3.9	15	335	4.5	283	1175	24.1	11	1243	0.9	9	1182	0.72
10^5	6	621	1.0	3	487	0.6	8	573	1.4	34	523	6.5	0	1034	0.0	1	1126	0.08
10^4	0	451	0.0	0	302	0.0	0	276	0.0	17	527	3.2	0	1046	0.0	0	1258	0.00
10^3	0	435	0.0	0	305	0.0	0	329	0.0	2	1190	0.2	0	1123	0.0	0	1054	0.00
10^2	0	226	0.0	0	216	0.0	0	304	0.0	3	1670	0.2	0	275	0.0	0	249	0.00

In part A NH4104 used as the donor strain and AB266 as recipient and growth was in PB. Frequencies of Lac⁺ cells were determined by diluting and plating on EMB latose agar plus streptomycin. In part B NH4104 used as the donor strain, AB266 as recipient; in part C NH4104 used as the donor strain and MSU2002 as recipient. In both parts, B and C, growth was in M medium and the frequencies of Lac⁺ cells were determined by diluting and plating on tetrazolium plates plus streptomycin.

of the formerly associated lac^+ allele, leading to a haploid Lac⁻Hfr.

(3) crossing over between F factor and bacterial chromosome resulting

in a lac^-/lac^- homozygous merodiploid. Twenty two segregated Lac⁻

phenotypes were isolated from an overnight culture of MSU2001 and

were checked for their F status. It was found that 9 of them were lac^-

(F^-), 13 of them were lac^-/lac^- (F^+), and none of them was Hfr. This

explains why the segregation of Lac⁻ phenotypes in strain MSU2001 is

higher than that found in F42/JC1553.

Measurements of rate of loss of F factor in F42/JC1553. Since

spontaneous loss of the F factor occurs in E. coli populations, some

knowledge of its frequency is obviously important in understanding the

origin of F⁻ cells in motility medium swarms. Few careful estimates

of spontaneous loss of fertility factor have been reported. Jacob and

Adelberg (1959) first reported that the segregation rate of Lac⁻ pheno-

types from a $lac^-/F'-lac^+$ heterogenote is 10^{-3} per cell division.

Palchoudhury and Iyer (1969) reported a spontaneous loss frequency of

the F factor of about 0.0064% in a RecA strain. However their measure-

ment was made at a relatively high cell concentration (2×10^6/ml) where

reinfection may occur. Stadler and Adelberg (1972) reported a high

rate of F factor loss when Rec^+ cultures were grown at 43°C and at a

low cell density (less than 5×10^4/ml). Besides, they also found that

the rate of episome loss is dependent on the genetic background and

no elimination of fertility factor was observed at 37°C in the strains

they used. Since F42/JC1553 is a RecA strain and has a different genetic background from the strains used in the studies mentioned above, it was essential to obtain a careful measurement of spontaneous F factor loss in this strain.

It is difficult to measure the rate of F factor loss directly in solid or semisolid media. Therefore this measurement was made in a liquid medium --- M medium (which contains all the components of motility medium except gelatin and agar, and will provide the maximal similarity to the motility medium), and use this figure to estimate the rate of F loss in motility medium.

Since lactose and 2,3,5-triphenyl tetrazolium chloride were added into the motility medium in the studies of swarming, it was important to test the effects of these substances on F loss. Hence in the measurements of F loss, strain F42/JC1553 was grown in two kinds of media: M medium and M medium plus lactose and tetrazolium salt, M(TZ), separately. Any difference in the rate of F loss between such cultures will illustrate that tetrazolium salt can alter the rate of F loss.

If the rate of spontaneous loss of the F factor is constant and the Lac⁻ phenotypes are not induced by the plating on tetrazolium plates (if induction occurs the variance of Lac⁻ cells in different plates should be low), it can be treated in the same way as a bacterial mutation. Two methods that have been employed in the measurement of mutation are the median culture method and negative culture method.

In the former, mutation rate is calculated from the number of mutants in the median culture of a population of similar cultures. In the second method, the mutation rate is calculated from the frequency of cultures containing no mutations in a population of similar cultures. In using either method to estimate the F factor loss, it is necessary that populations be kept at low cell densities (less than $5x10^4$/ml) to prevent reinfection.

Rate of F factor loss in median culture method. Table 4 illustrates the calculation of the rate of F loss by the median culture method. From the chart published by Lea and Coulson (1949), the mean number of mutations per culture in both media was calculated and the mutation rate in both M medium and M(TZ) medium were estimated to be $4.08-4.70x10^{-4}$ and $4.17x10^{-4}$ F-factor losses per cell division respectively. We conclude that the presence of tetrazolium salt and lactose in the M medium does not induce a higher rate of F factor elimination.

Rate of F factor loss in negative culture method. Experiments in which the mutation rate is estimated by the negative culture method are summarized in Table 5. The calculated mutation rates ranged from $7.4x10^{-4}$ to $6x10^{-3}$ losses per cell division; the overall average is $2.8x10^{-3}$.

The mutation rate calculated by the median culture method is about 1/6 that calculated by the negative culture method. One interpretation of the lower mutation rate estimated by the median culture method is