SEX HAIR (F-PILI) MUTANTS OF E. COLI

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In this communication we describe the isolation of a class of mutants of male bacteria which restrict the infection of RNA bacteriophage f2. The mutants are infected normally by the male-specific DNA-containing phage f1 and by the serologically distinct RNA phage Qβ. These mutants, obtained by nitrosoguanidine treatment of E. coli (HfrC), produce normal levels of sex hair (F-pili) and in fact readily adsorb f2. They appear to be blocked at a later extracellular stage of infection - possibly at a transport or injection step (Valentine and Wedel, 1965). We speculate that the sex hair of these strains may be functionally altered by a mutation.

Results and Experimental

Isolation of f2 Resistant Strains. E. coli HfrC, strain W1895, was treated with 100 μg/ml N-methyl-N'-nitroso-N-nitrosoguanidine (NTG) as described by Adelberg et al. (1965). After treatment with NTG the cells were spread on EMB-0 plates (Zinder, 1960) to give about 100 colonies per plate and incubated at 37°C for 15-17 hours. The colonies were next sprayed from a chromatographic spray jar with a fine mist of a lysate (10^{12} phage per ml) of RNA phage f2 (Zinder, 1960). After 3-5 hrs of further incubation at 37°C the f2 infected colonies were scored for phage resistance or sensitivity as illustrated in Fig. 1. About 900 mutants resistant to f2 were isolated by this procedure.

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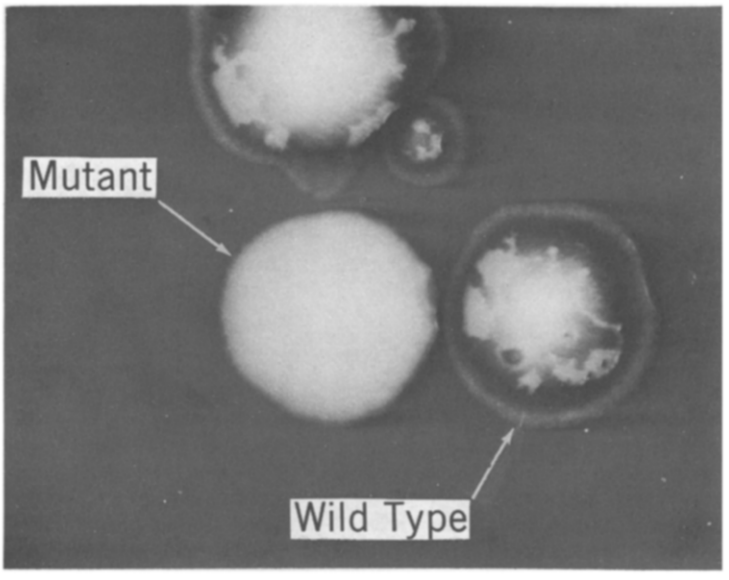

Fig. 1. Spray test for f2 resistant mutants. The virus (f2) has attacked the male colony (wild type) causing dye precipitation around the colony. The mutant colony is not affected. Method after Zinder, 1960.

Mutants Resistant to f2 but Sensitive to f1. The f2 resistant mutant strains were next tested for sensitivity to male-specific phage f1 by spotting a loopful of phage lysate on a lawn of each mutant strain. Only two of approximately 900 mutants showed sensitivity to f1. One of these mutants is illustrated in Fig. 2 below. Most mutants were simultaneously resistant to both f1 and f2. The growth rate and other properties of the mutant strain were found to be comparable to the parent stock. It was of some interest that RNA-bacteriophage Qβ (Overby et al., 1966) formed plaques on the mutant host (Fig. 2). This finding is described in more detail below.

Qβ: A Naturally Occurring "Host Range" Mutant. We were surprised to find that RNA-phage Qβ plated with high efficiency on the mutant host (Fig. 2). Several other viral strains including MS-2 and R-17 did not form plaques. In one sense Qβ may be regarded as a naturally occurring "host range" mutant against this particular host. It should be pointed

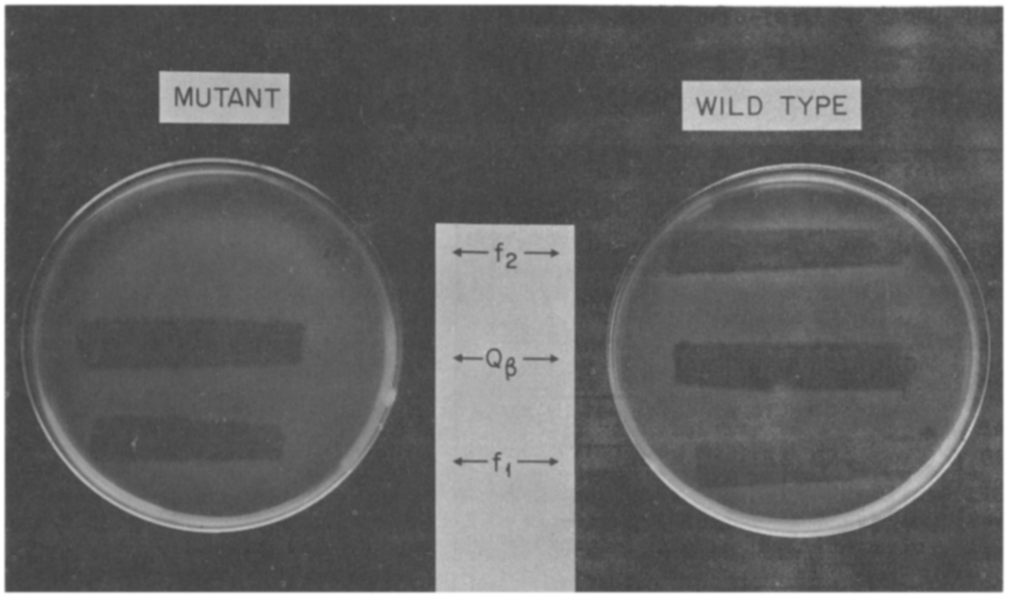

Fig. 2. Phage lysis test showing sensitivity of mutant male strain to filamentous phage f1 and RNA phage Qβ; note resistance to RNA phage f2.

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out that Qβ is thought to belong to a distinct class of RNA-containing coliphages - varying in serotypes and even base ratio from f2 (Overby, et al., 1966). Our findings with Qβ have encouraged us to look for a set of host range mutants of f2; this work is in progress.

Nature of the Lesion in the Mutant. We first tested the mutant strain to see if it synthesized F-pili by using the filtration assay for F-pili as described previously (Ippen and Valentine, 1965). This data is presented in Fig. 3. It is apparent that the mutant strain produces normal levels of F-pili as measured by phage adsorption. Electron micrographs confirmed this finding.

The penetration step of infection was next measured (Valentine and Wedel, 1965). As shown in Fig. 3, little viral RNA penetrated the cells. We conclude that RNA phage infection in the mutant strain is blocked at or before the penetration step but after the adsorption step (Valentine and Wedel, 1965). These results are discussed below.

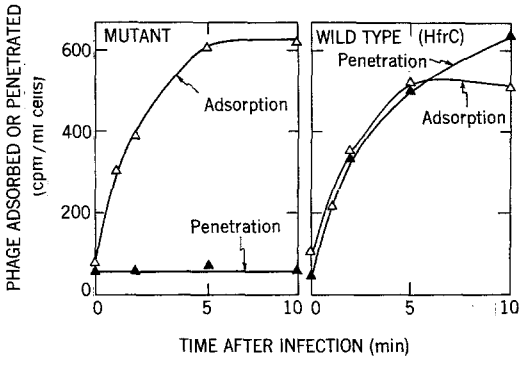

Fig. 3. Mutant adsorbs phage but the later RNA penetration step is blocked. Adsorption and penetration of phage was followed using radioactive (P^{32}) phage as described previously (Ippen and Valentine, 1965; Valentine and Wedel, 1965). The absolute quantities of radioactive phage adsorbing and penetrating are not related since different quantities of phage were used for the two assays.

Discussion

We originally intended to isolate F-pili mutants of male strains of E. coli. It seemed reasonable to screen the mutants against both varieties of male-specific phage (f1 and f2) since both phages were known to use the F-pilus in different ways (Caro and Schnös, 1966; Ippen and Valentine, 1966). In this way we thought it might be possible to isolate mutant males with altered F-pili which adsorbed or penetrated one phage but not the other. The data presented above indicate that the present set of mutants belongs to this class - adsorbing but not penetrating f2. It should be pointed out that we have not entirely proven that extracellular RNA penetration is blocked; it is possible that the restriction for RNA phage infection occurs once the RNA has penetrated the cytoplasm of the host; for example, the viral RNA might be hydrolyzed as fast as it entered the host. However, if hydrolysis required more than two minutes to complete, then some accumulation of RNA would be expected to occur; as seen from Fig. 3 (mutant) little if any RNA accumulation in the host is observed.

We are continuing to study the nature and mechanism of the restriction but our working model is that the F-pili of the mutant are altered. The finding that the RNA-phage Qβ is able to grow on the mutant would appear to substantiate this view.

Studies of mutants of the type described here may aid in the elucidation of the mechanism of F-pili action. This is one of the major goals of the present work.

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