Selection of Mutant Bacterial Sex Factors Determining Altered Sex Pili

By G. G. MEYNELL AND EVA AUFREITER

Guinness-Lister Research Unit, Lister Institute, Chelsea Bridge Road, London, S.W.1

(Accepted for publication 18 September 1969)

Bacterial sex factors determine the synthesis of sex pili that act as receptors for donor-specific phages, so that many sex factors can be conveniently identified by the phage-sensitivity of their hosts (see Meynell, Meynell & Datta, 1968). Gross differences distinguish the two major groups of F-like and I-like pili and their factors, while minor differences are evident within the F-like group, as with the drug-resistance factor, R100-1, and the plasmid, F₀-*lac* (Nishimura, Ishibashi, Meynell & Hirota, 1967; Lawn, Meynell, Meynell & Datta, 1967; Meynell *et al.* 1968). However, for phage sensitivity to be generally useful as a genetic marker, one must be able to select mutant sex factors that confer sensitivity to only one class of phage, e.g. to isometric RNA phage but not to filamentous DNA phage. Valentine and his colleagues have shown that such mutants exist in mutagenized Hfr cultures of *Escherichia coli*, by infecting with RNA phage, plating for isolated colonies, spraying with the same phage and picking unlysed colonies (see Valentine, Silverman, Ippen & Mobach, 1969). Only a minute proportion (2/900) of such colonies contain mutant factors (Silverman, Mobach & Valentine, 1967), because phage resistance less often arises in this way than from poor expression of the pilus-forming ability of parental factors or from a complete mutational block in pilus synthesis. We have therefore tested an alternative method of selection which appears more efficient. It depends on the fact that, of the three classes of phage-resistant cell in the culture—unexpressed parental type, blocked mutant and the mutants to be selected—only the last will be phage-resistant yet able to synthesize sex pili and to transfer their sex factors by conjugation. A mutagenized donor culture is accordingly treated with phage, mated and the factors acquired by the recipient examined.

An overnight culture of *Escherichia coli* strain 24 carrying the de-repressed *fi*+ (F-like) R factor, R1drd-16 (Meynell & Datta, 1967) was treated with 0.4 M-ethylmethane sulphonate for 20 min. at 37°, diluted 1/100 into 10 ml. broth and grown overnight without shaking. Next morning 10¹⁰ particles of the RNA F phage, MS2, were added. After allowing 30 min. at 37° for adsorption, the infected culture was diluted 1/100 in broth, incubated overnight and then again diluted 1/100 in broth and incubated overnight to encourage pilus formation and consequent killing of cells with parental factors. The second stationary phase culture was diluted 1/20 in broth, incubated on a turntable for 2 hr at 37° and mixed with equal numbers of the recipient strain, *E. coli* 712, and left overnight at 37°. The recipient was finally isolated by streaking on glucose-salts agar containing the appropriate antibiotic and growth factors. The colonies obtained in two independent experiments were purified and tested for sensitivity to phage MS2, the unrelated RNA phage, Q β , and the DNA phage, fd.

Some colonies were sensitive to all three phages like the parental strain; others were resistant; and about 50 % were mutants resistant to MS2 but sensitive to Q β and fd.

Electron microscopy showed that, although the mutants appeared resistant to MS2 in spot tests, their pili could adsorb the phage, albeit far less efficiently than the pili of their parent. These mutants may therefore resemble the type A mutants of Valentine *et al.* (1969). Plate 1 shows a mixture of mutant and parental pili after addition of a high multiplicity of phage MS2. To prepare the specimens a mutant and a parental culture were mixed and phage added. After 20 min. at room temperature, the suspension was fixed with formalin and the cells collected on a membrane filter, from which they were transferred to an electron microscope grid and stained with uranyl acetate. As Pl. 1 shows, the parental pili were heavily coated with phage whereas relatively few particles attached to the mutant pili. Moreover, the mutant pili had a conspicuously abnormal appearance in that they formed bundles and showed numerous vesicular structures resembling the 'knobs' previously seen on the tips of both F and I sex pili (Lawn, 1966; Meynell & Lawn, 1967).

Despite their abnormal appearance, these mutant pili evidently allowed conjugation and gene transfer, since this provided the means by which the mutants were isolated. Mating experiments showed that 2 to 25 % and 0.7 to 3 % of cells in the two mutant strains could transfer their drug resistance in 20 min., compared to 50 % for their parent. The abnormal morphology of the mutant pili therefore suggests that the alteration in the pilus protein that leads to poor adsorption of phage MS2 also results in structural instability of the pilus.

The electron microscopy was kindly undertaken by Dr A. M. Lawn.

REFERENCES

- LAWN, A. M. (1966). Morphological features of the pili associated with R⁺F⁻ and R⁻F⁺ bacteria. *J. gen. Microbiol.* **45**, 377.
- LAWN, A. M., MEYNELL, E., MEYNELL, G. G. & DATTA, N. (1967). Sex pili and the classification of sex factors in the Enterobacteriaceae. *Nature, Lond.* **216**, 343.
- MEYNELL, E. & DATTA, N. (1967). Mutant drug resistance factors of high transmissibility. *Nature, Lond.* **214**, 885.
- MEYNELL, E., MEYNELL, G. G. & DATTA, N. (1968). Phylogenetic relationships of drug-resistance factors and other transmissible bacterial plasmids. *Bact. Rev.* **32**, 55.
- MEYNELL, G. G. & LAWN, A. M. (1967). Sex pili and common pili in the conjugational transfer of colicin factor Ib by *Salmonella typhimurium*. *Genet. Res.* **9**, 359.
- NISHIMURA, Y., ISHIBASHI, M., MEYNELL, E. & HIROTA, Y. (1967). Specific piliation directed by a fertility factor and a resistance factor of *Escherichia coli*. *J. gen. Microbiol.* **49**, 89.
- SILVERMAN, P. M., MOBACH, H. W. & VALENTINE, R. C. (1967). Sex hair (F-pili) mutants of *E. coli*. *Biochem. biophys. Res. Commun.* **27**, 412.
- VALENTINE, R. C., SILVERMAN, P. M., IPPEN, K. A. & MOBACH, H. (1969). The F-pilus of *Escherichia coli*. *Adv. microbiol. Physiol.* **3**, 1.

EXPLANATION OF PLATE

PLATE 1

A mixture of mutant (M) and parental (P) pili mixed with phage MS2. The vesicular structures associated with the mutant pili are marked by arrows.