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[1] Dodds, E. C., Folley, S. J., Glascock, R. F., and Lawson, W., *Biochem. J.*, **68**, 161 (1958).
[2] Glascock, R. F., and Hoekstra, W. G., *Biochem. J.*, **72**, 673 (1959).
[3] Ficq, A., *Arch. Biol.*, **66**, 509 (1955).
[4] Budy, A. M., *Arch. intern. Pharmacodynamie*, **103**, 435 (1955).
[5] Rengier, I., *Proc. Soc. Exp. Biol. Med.*, **95**, 506 (1957).

BACTERIOLOGY

Interspecific Hybridization among *Mycobacteria*

Experiments conducted in the first part of this work showed that a high degree of streptomycin resistance can be transmitted to *M. phlei* when a streptomycin-sensitive strain of this micro-organism and a streptomycin-resistant *BCG* strain of *M. tuberculosis* are cultured together in 'Tween' liquid medium.

Resistance to 50 μgm. streptomycin per ml. (S^r) was selected as marker in the *BCG* strain; the markers selected for *M. phlei* were pigment formation (Pig^+) and the capacity for growth on plain agar medium (Ag^+). The *BCG* strain may thus be described as S^r Pig^- Ag^-, while *M. phlei* is S^s Pig^+ Ag^+. Colonies of the new organism were picked from a selective medium: tryptose agar containing 50–1,000 μgm. streptomycin per ml. on which neither parent organism could grow when planted alone. Their inability to grow on this medium in 30 or more days served as control. The frequency of resulting streptomycin-resistant organisms was estimated to be 1–10 per 10^6 *M. phlei* units. Similar results were obtained in four experiments.

One of the strains obtained in the course of these experiments deserved special attention as it resembled the so-called 'atypical' acid-fast chromogenic *Mycobacteria*. This hybrid, in addition to high streptomycin resistance, showed yellow pigmentation (both in the dark and in the light) which, in contrast to that of the parental *M. phlei*, turned orange on prolonged cultivation. At 37° C. the strain grew on tryptose agar, as well as on 'Tween' agar or Loewenstein's medium within 5–12 days; at room temperature growth appeared slowly, in 12–20 days. No cord formation was seen. A high degree of pleomorphism and instability of traits other than the selected markers were found to be characteristic.

Since streptomycin was the only selected marker of the *BCG* strain used in these experiments, it was not possible to make any recombination analysis, or to assess the importance of a cell-to-cell contact (conjugation) in the transfer of streptomycin resistance.

Experiments were, therefore, carried out in order to shed more light on the mechanism of transfer: *M. phlei* was grown in the culture filtrate of the *BCG* strain; and *BCG* in the culture filtrate of *M. phlei*. Cell-free filtrates of *M. phlei* were never observed in these experiments to bring about changes in the *BCG* strain. Sterile culture filtrates of the *BCG* strain, on the other hand, conferred the streptomycin resistance to *M. phlei* cells. This could be repeated in three experimental series. The frequency, however, was 10^2 lower, that is, 1–10 per 10^8 *M. phlei* units. These findings indicate that the transfer of streptomycin resistance does not necessarily require cells as donors, and thus transduction may be the actual mechanism of transfer. (In the trials already carried out deoxyribonuclease treatment of the filtrate has not affected significantly its activity.)

These experiments, both those in which a *BCG* culture and those in which cell-free filtrates of a *BCG* culture were used as donors, disclosed also that: (1) The streptomycin resistance of descendant organisms increased to a level of more than 1,000 μgm. per ml. as against a level of 50 μgm. per ml. of the parental *BCG* strain of *M. tuberculosis*. (2) This high level of streptomycin resistance was attained in a single step, while the parent strain of *BCG* acquired the much lower resistance in four or five distinct steps. (3) In addition to this, a considerable portion of the resulting variants showed on isolation dependence on streptomycin. (4) Evidence was also obtained supporting the assumption that the transfer of hereditary traits is uni-directional, that is, from *BCG* strain of *M. tuberculosis* to *M. phlei*.

The results of the work described here appear to bear a resemblance to the findings made in the course of investigations of genetic transfer in various species of bacteria[1–5]. Studies on genetic transfer together with certain recently reported observations with respect to mutation[6–9] may, it is hoped, some day give an answer to the origin of atypical mycobacteria.

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[1] Zinder, N. D., and Lederberg, J., *J. Bacteriol.*, **64**, 679 (1952).
[2] Luria, S. E., and Burrows, J. S., *J. Bacteriol.*, **74**, 461 (1957).
[3] Leidy, G., Hahn, E., and Alexander, H. E., *J. Exp. Med.*, **104**, 305 (1956).
[4] Miyake, T., and Demerec, M., *Nature*, **183**, 1586 (1959).
[5] Terada, M., Abstr. Seventh International Congress of Microbiology, Stockholm (1958).
[6] Tarshis, M. S., *Amer. Rev. Tubercul.*, **78**, 921 (1958).
[7] Tirunarayanan, M. O., Vischer, W. A., and Bruhia, H., *Schweiz. Z. Path. Bakt.*, **21**, 964 (1958).
[8] Mayer, R. L. Eisman, P. C., Gisi, T. A., and Konopka, E. A., *Amer. Rev. Tubercul.*, **77**, 694 (1958).
[9] Di Marco, A., Ghione, M., and Zavaglio, V., *Giornale di Microbiologia*, **1**, 266 (1956).

Role of *Hfr* Mutants in *F*+ × *F*− Crosses in *Escherichia coli* K12

Genetic recombination between mutant strains of *Escherichia coli* strain *K*12 is governed by a system of mating types[1,2]. The first two mating types to be discovered were called *F*+ and *F*−. In crosses the *F*+ parent donates genetic material to the *F*− parent. The progeny are predominantly *F*+. At the same time it was discovered that the *F* factor was infectious, that is, if *F*+ and *F*− strains were mixed and the organisms of the *F*− strain re-isolated, a proportion had become *F*+. It was therefore possible that the *F*+ state of the progeny was secondary and that any *F*-zygotes became infected by other *F*+ cells.

Then came the discovery of mutant strains of a new mating type known as high-frequency recombining (*Hfr*) strains[1,3]. Whereas *F*+ strains crossed with *F*− strains gave only one recombinant per