"RESISTANCE TRANSFER FACTOR" AN EPISOME IN ENTEROBACTERIACEAE

T. Watanabe and T. Fukasawa

Department of Bacteriology

School of Medicine, Keio University, Tokyo, Japan

Received November 29, 1960

Many strains of Shigella and E. coli isolated in Japan in recent years are resistant to more than two drugs of streptomycin (Sm), chloramphenicol (Cm), tetracycline (Tc) and sulfa drugs (Su). Ochiai et al. (1959) and Akiba et al. (1960) found that multiple drug resistance of these strains can be transferred to sensitive strains of Shigella and E. coli by growing them together. They also found that cell-free filtrates of donor cultures can not transfer resistance and assumed that cell-to-cell contact is essential for its transfer. Mitsuhashi et al. (1960) reported that F-factor is not required for the transfer of multiple drug resistance in E. coli strain K-12. Nakaya and Nakamura (1960) reported that multiple drug resistance can be transduced by phage P1kc. We have also conducted a series of genetic studies on the multiple drug resistance. Our results are briefly reported here and will be published in detail elsewhere.

EXPERIMENTAL RESULTS

Transfer of resistance factors by mixed culture. Shigella flexneri 2b strain 222 resistant to Sm, Cm, Tc and Su and E. coli K-12 strains were used. The recipient cells which

acquired resistance factors were detected on proper selective media containing each drug in proper concentrations. All of the resistance factors were found to be transferred together and the recipients thus made resistant were all able to transfer the resistance further to other sensitive strains. The frequency of transfer was about 10^{-3} per donor cell in mixed culture for 1 hr. UV-irradiation of donor cells increased the frequency of transfer 10 to 100 times higher. Kinetic studies with blendor treatment (Jacob and Wollman, 1955) revealed that all of the resistance factors are transferred at about 15 minutes after conjugation which takes place almost instantly after donor and recipient are mixed. Furthermore, when a small number of donor cells was mixed with a large number of recipient cells, a majority of the recipient cells acquired resistance factors faster than the multiplication of the recipient cells, indicating that the resistance factors replicate autonomously.

Acridine elimination and spontaneous loss of resistance factors. The resistance factors were eliminated by treatment of the cells with acriflavine and acridine orange using the conditions of Hirota (1960) for F-factor. It was found that the resistance factors are eliminated together with a frequency as low as 4.1% in acriflavine and even lower in acridine orange. UV-irradiation of the resistant cells before acridine treatment increased the frequency of elimination upto almost 100%. The resistance factors were found to be lost also spontaneously in low frequencies with a penicillin screening method using a mixture of penicillin and either Cm or Tc. Complete loss as well as segregated loss was found. In the segregated loss, either Tc-resistance alone or

Sm-, Cm- and Su-resistance together was lost. All of these segregants were able to transfer their remaining resistance factors by conjugation.

Changes of the cells which received resistance factors. The cells which received resistance factors by conjugation did not show any other changes than drug resistance and its transferability. They did not produce colicin as far as examined and F^- cells of K-12 which received resistance factors did not acquire the ability to transfer their chromosomal markers to other F^- strains.

Transduction of resistance factors. We found that resistance factors can be transduced in K-12 with P1kc and in Salmonella typhimurium strain LT-2 with P-22. The resistant transductants of both K-12 and LT-2 were found to show segregation of resistance factc In K-12, 93% of the transductants had all of the four resistance factors and 5% Sm-, Cm- and Tc-resistance and 2% were resistant to Tc alone. All of them were able to transfer their resistance factors by conjugation. Linkage relationships of the resistance factors to host chromosomal markers was studied with transduction in K-12 strains but the attempts have been so far unsuccessful. In transduct with P-22, resistance factors were found to segregate into Sm, Cm plus Su (86.6%), Tc alone (12.8%) and Sm plus Su (0.6%). A majority of the transductants of LT-2 was unable to transfer their resistance to other sensitive strains by conjugation, although the LT-2 cells which received by conjugation either Tc-resistance alone or the resistance to Sm, Cm and Su were able to transfer their resistance by conjugation. Only one exceptional transductant with Tc-resistance (0.6% of the total transductants) was able to transfer its resistance factor. To the transductants of LT-2 with a

part of the resistance factors can be transferred the other resistance factors by conjugation with Shigella strains which carry complementary resistance factors. The strains thus made resistant to all of the four drugs were found to transfer by conjugation only the resistance factors received by conjugation and not any other resistance factors obtained by transduction.

DISCUSSION

It is now obvious that the transmissible resistance factors of Enterobacteriaceae very much resemble the episomes (Jacob and Wollman, 1958; Jacob et al., 1960). The resistance factors were found to replicate autonomously through the experiments on their transfer by conjugation and also on their elimination by acridines. The transferred resistance factors again replicate autonomously in the recipient cells. The transduced resistance factors in K-12 all replicate autonomously but a majority of the resistant transductants of LT-2 is unable to transfer their resistance factors by conjugation. This inability of transferring resistance factors might be due to the failure of LT-2 transductants to conjugate in spite of their autonomous replication. This possibility can be ruled out because the resistance factors transferred by conjugation to these transductants can be further transferred by conjugation but the transduced resistance factors can not. The transduced resistance factors in LT-2, therefore, must be in integrated state, suggesting that the resistance factors had originally come from bacterial chromosomes. If each resistance factor were an episome, it is very likely that the transduced resistance factors also undergo "transfer induction" in the recipient cells (Arber, 1960). We assume,

therefore, that each resistance factor is not a transmissible factor in itself but that the resistance factors are carried by some transmissible factor. If the resistance factors had originated from bacterial chromosomes, there must have been a stage of attachment of the above transmissible factor to host chromosomes. This assumption leads to the hypothesis that this factor is an episome. This hypothetical episome can not be a phage because it does not kill the cell in which it replicates autonomously and also because cell-free filtrates of donor cultures can not transfer resistance. The transfer of resistance factors by this factor is very much like F-duction (Jacob et al., 1960). It apparently does not induce colicin production and can be transferred by conjugation without the aid of F-factor (Fredericq, 1957). However, it does not give fertility to the F^- cells of K-12 but this failure might be due to the heterogeneity between this factor and the chromosomes of K-12. We found that this factor and F-factor repress each other (to be reported later), indicating that this factor has close similarity to F-factor. We propose to refer to this factor as "resistance transfer factor (RTF)" tentatively. The resistance factors are possibly attached to this episome in the following sequence assuming from the data of transduction;

Su----------Sm---------Cm---------Tc---------RTF.

SUMMARY

The transfer of multiple drug resistance in Enterobacteriaceae was studied by conjugation and transduction. The resistance factors were found to be carried by an episome ("resistance transfer factor (RTF)").

REFERENCES

Akiba, T., Koyama, T., Isshiki, T., Kimura, S. and Fukushima, T., Nihon Iji Shimpo, 1866, 46(1960) (in Japanese).

Arber, W., Virology, 11, 273 (1960).

Fredericq, P., Ann. Rev. Microbiol., 11, 7(1957).

Jacob, F., and Wollman, E. L., C. R. Acad. Sci., 240, 2449 (1955).

Jacob, F., and Wollman, E. L., C. R. Acad. Sci., 247, 154 (1958)

Jacob, F., Schaeffer, P., and Wollman, E. L., Microbial Genetics (Tenth Symp. Soc. Gen. Microbiol.), 67 (1960).

Hirota, Y., Proc. Natl. Acad. Sci. U. S., 46, 57(1960).

Mitsuhashi, S., Harada, K., and Hashimoto, H., Japanese J. Exp. Med., 30, 179 (1960).

Nakaya, R., and Nakamura, A., Presented at the 13th Meeting of the Kanto Branch of the Society of Japanese Bacteriologists (1960).

Ochiai, K., Yamanaka, T., Kimura, K., and Sawada, O., Nihon Iji Shimpo, 1861, 34 (1960) (in Japanese).