SHORT COMMUNICATION

R FACTOR INSTABILITY IN THE ACRIFLAVINE-SENSITIVE MUTANT OF *ESCHERICHIA COLI* K 12

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It was reported previously that plasmid F-*gal*⁺ in *Escherichia coli* K 12 cells is eliminated at a high frequency under various conditions when the host cells contained the mutated allele *acrA* than when it contained the wild type allele *acrA*⁺ (Nakamura 1974). The *acrA* mutation, which is mapped at min 11 on the *E. coli* chromosome (Nakamura *et al.* 1975), leads to deficiency of a membrane protein referred to as *acrA*⁺ protein (Nakamura and Suganuma 1972; Nishimukai *et al.* 1973). Therefore, the above observation was interpreted as that the *acrA*⁺ protein of the host cell membrane is responsible for the replication of F factor (Nakamura 1974).

A question arised here whether the membrane protein is specific for the F-factor's replication. The present report deals with the effects of the *acrA* mutation on the stability of the two kinds of drug resistance factors.

All the bacterial strains used were derivatives of *E. coli* K 12. Strains N43 (F⁻ *lac acrA*) and N90 (F⁻ *lac acrA*⁺) were described previously (Nakamura 1965). R⁺ strain JE2100 (R₁₀₀₋₁⁺ *fi*⁺ *drd/lp-s*) and J53 (R₆₄₋₁₁⁺ *fi*⁺ *drd/pro met*) were kindly supplied by Dr. Tomoeda. Plasmid tranfers were performed by conjugation of JE2100 with N43 and N90 for R₁₀₀₋₁, and J53 with N43 and N90 for R₆₄₋₁₁. The resulting representative strains were referred to as N2529 for R₁₀₀₋₁/N43, N2538 for R₁₀₀₋₁/N90, N2557 for R₆₄₋₁₁/N43, and N2550 for R₆₄₋₁₁/N90. Plasmid R₁₀₀₋₁ conferred resistance to tetracycline (TC), chloramphenicol, streptomycin and sulfanil amide, and R₆₄₋₁₁ conferred TC and streptomycin. Selection, thus, was done for resistance to TC (25 µg/ml). Typing of the plasmids was done by using phages MS2 for R₁₀₀₋₁ (F-type) and Ifl for R₆₄₋₁₁ (I-type), kindly supplied by Dr. Tomoeda.

Broth medium PGY was prepared as described previously (Nakamura 1965).

Preliminary experiments indicated that the R factors were significantly cured in the presence of acridine dyes such as acriflavine (AF), proflavine, quinacrine, acridine orange, 9-aminoacridine, and acridine: it was effective in lower concentrations when hosted by the *acrA* cells than when hosted by the *acrA*⁺ cells.

Freshly grown R⁺ cells were inoculated into PGY (pH 7.8) media containing 0.5 and 1.0 µg AF/ml and shaken at 37°C. Plasmid curing was determined by the replica-plating method (Tomoeda *et al.* 1968) using PGY (pH 7.4) agar containing 25 µg TC/ml.

Fig. 1. Curing of R_{100-1} (A) and R_{64-11} (B) during the cell division of *acrA* and *acrA*⁺ strains in the presence of AF. Freshly grown N2529 (open) and N2538 (closed) (in A) and N2557 (open) and N2550 (closed) (in B) were inoculated into PGY (pH 7.8) media containing no ($\circ$, $\bullet$), 0.5 ($\triangle$, $\blacktriangle$) and 1.0 ($\square$, $\blacksquare$) μ g AF/ml. The R^+ cells were scored at intervals during incubation at 37°C. Growth rates (doublings per hr) were 3.8 to 1.3 for the *acrA* strains and 3.7 to 3.3 for the *acrA*⁺ strains, depending upon the AF concentration in the medium.

The total colonies counted per plate were 100 to 400. Fig. 1A indicates that the R_{100-1} factor of N2529 was cured as the cell generation went by, remarkably in 1.0 μ g AF/ml and less in 0.5 μ g AF/ml. Fig. 1B shows that the R_{64-11} factor of N2557 also was effectively eliminated in the presence of 0.5 and 1.0 μ g AF/ml but that of N2550 was not. Therefore, it can be concluded that the R factors hosted by the *acrA* cells, either of type F or I, are eliminated in the presence of AF during the active cell division.

Another approach was to see whether R^+ cells at stationary growth phase were cured in the presence of AF. Strains N2529 and N2538 were cultured in PGY (pH 7.4)

medium for 24 hrs at 37°C. The cultures thus at the full growth were pipetted into a series of tubes containing graded concentrations (1-6 µg/ml) of AF. After incubation for 24, 48, and 72 hrs, R⁺ cells were counted. It was shown as the result that the R factor of N2529 was eliminated more remarkably than that of N2538. On the other hand, the N2529 cells were significantly cured even in the AF-free medium by prolonged incubation but the N2538 cells were not. Therefore, nevertheless the *acrA* cells bind more AF than the *acrA*⁺ cells (Nakamura 1966, 1974), unstability of R factor in the former cells may be due to the membrane defect. The difference in the stability of R factors between *acrA* and *acrA*⁺ strains, however, seems to be smaller than the case of F-*gal*⁺ factor (c.f. Nakamura 1974).

The R factor curing occurred either in the exponentially growing phase or in the stationary phase. According to unpublished data, the F-*gal*⁺ factors also were cured at the stationary phase in the presence of AF. Therefore, it is suggested that the replication and residence of R factors, just as of the F-*gal*⁺ factors, are controlled by the *acrA* protein of the plasma membrane.

Tomoeada *et al.* (1968) have reported that R factors are eliminated in the presence of sodium dodecylsulfate at the stationary growth phase but not during the active cell growth. They also found that the R⁺ cells at the stationary phase are more sensitive to sodium dodecylsulfate than the R⁻ cells at the same phase. However, in the present experiments, no significant difference in the AF sensitivity was observed between the R⁺, R₁₀₀₋₁ or R₆₄₋₁₁, and R⁻ cells. Furthermore, the mutated *acrA* gene does not influence TC sensitivity of the cells. It still remains open why curing of these plasmids occurs at the stationary growth phase in the presence of AF.

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