Genetic Determination of Sensitivity to Salt Hypertonicity in *Escherichia coli* K12

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Many Gram-negative non-halophilic bacteria, including *Escherichia coli*, are plasmolysed when suspended in high salt solutions. This observation has been interpreted as evidence for the presence of an osmotic barrier in the plasma membrane, impermeable to salt (Larsen, 1962). The phospholipid bilayer constituting the plasma membrane must interfere with the penetration of Na⁺ ions. However, any fundamental differences in ion permeability between bacterial strains must be determined by the membrane proteins specific to the strain (see Koh, 1975).

An acriflavine (AF) sensitivity mutation (*acrA*) lying at min 10 on the *E. coli* chromosome (Nakamura, Tojo & Greenberg, 1975) determines the loss of a protein (referred to as *acrA*⁺ protein) of the plasma membrane (Nishimukai *et al.*, 1973). The purpose of the present work was to examine whether the *acrA*⁺ protein is responsible for cell sensitivity to salt.

All strains used were derivatives of *E. coli* K12. The genotypes of N43 and N90 are *acrA* and *acrA*⁺ (AF-resistance) respectively (Nakamura, 1965). Suppressed AF-resistant revertants, N2307, N2308 and N2309, and a true revertant N2310 of N43 were described previously (Nakamura, 1974). The salt-resistant mutant N2800 of N43 was spontaneous and was isolated after 11 transfers in PGY medium with NaCl added to 5.3 % (w/v).

Basal medium PGY, which contained 0.3 % NaCl, was prepared as described previously (Nakamura, 1965). The pH was adjusted to 7.8 when the medium contained AF and to pH 7.4 without the drug. PGY medium of double strength was used when made hypertonic with NaCl, KCl or sucrose. For solid media, powdered agar was added to 1.5 % (w/v).

Freshly grown cells of strains N43 and N90 were inoculated into PGY medium containing 5.3 % NaCl and incubated at 37 °C. Samples were withdrawn at regular intervals and plated on to PGY agar for determination of the viable count. Figure 1 shows that strain N43 is more sensitive to salt hypertonicity than N90.

When strains N2307, N2308, N2309 and N2310 were plated on to PGY agar plates containing NaCl at varying concentrations (0.3 to 5.3 %) and compared with control strains N43, N90 and N2800, it was found that the AF-resistant revertants were also resistant to salt.

We then attempted to find out whether mutants selected for resistance to salt hypertonicity are also resistant to AF. Freshly grown cells of N2800 and N43 (as controls) were inoculated into PGY media pH 7.8 containing 2 or 5 µg AF ml⁻¹ and incubated at 37 °C. The results showed that N2800 was significantly more resistant to AF compared with N43. This strongly suggests that sensitivity to salt and AF is determined by the same gene, *acrA*.

An experiment was performed to ascertain whether resistance of *acrA*⁺ strains to high concentrations of salt is related to high osmotic pressure or whether it is specific to Na⁺ ions. When fresh cells of N43 and N90 were cultivated in PGY medium containing 1.2 M-sucrose, the latter strain had a significantly higher growth rate than the former. It can therefore be concluded that at least part of the salt resistance determined by the *acrA*⁺ membrane protein is an expression of resistance to high osmotic pressure.

The specificity of the *acrA*⁺ function for resistance to Na⁺ and AF was next examined. Overnight cultures of strains N43, N90, N2800 and N2309 were plated on PGY agar plates

Fig. 1. Salt sensitivity of *acrA* and *acrA*⁺ strains. Strains N43 (○) and N90 (●) were inoculated into PGY medium with NaCl at 5.3 % (—) or without NaCl (---).

containing varying concentrations [0.8 to 12.8 % (w/v)] of KCl. The results showed that bacterial sensitivity to KCl did not correlate with those for NaCl and AF.

The *acrA* gene determines sensitivity to basic dyes including AF (Nakamura, 1965), and its mutant allele directs an increased uptake of the dyes (Nakamura, 1966). It might be postulated that more Na⁺ ions penetrate through the membrane of the mutant than through that of the *acrA*⁺ strain in high salt solutions. This is in accordance with recent results which suggest that in *E. coli* and other non-halophilic bacteria, the intracellular machinery is protected from the high salt environment by the plasma membrane barrier (Larsen, 1962, 1967; Baley & Kusher, 1964; Measures, 1975). Therefore, *E. coli* cells will become hypersensitive when the *acrA*⁺ protein, which may be a component of the membrane barrier against salt, is lost by mutation.

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