RESISTANCE TO ACRIDINES AND THYMINELESS DEATH IN A MEC⁻ MUTANT OF ESCHERICHIA COLI

R.J. Pinney & M.J. Tribe, Microbiology Section, Department of Pharmaceutics, The School of Pharmacy, University of London, Brunswick Square, London, WC1N 1AX

0.92 moles per cent of cytosine bases in Escherichia coli strain K12 are 5-methylated (Vanyushin, et al., 1965). The methyl transferase responsible is coded by the mec^+ gene. No indispensable function has been ascribed to 5-MeC but the methylase does act as a modification enzyme and partially protects against the RN3 plasmid-mediated EcoRII(hspII) restricting nuclease. When received by us, E.coli strain 1100 mec^+ and its isogenic mec^- derivative (Hattman, et al., 1973) harboured the sex plasmid F, and "curing" of F from these strains by the classic acridine orange (AO) technique (Hirota & Iijima, 1957) showed that the minimum inhibitory concentration (MIC) of AO was approximately twofold higher for the mec^- than for the mec^+ strain. Increased resistance to AO was still observed in F-cured mec^- cell lines, and strain 1100 mec^-F^- was also shown to have a two fold higher MIC to aminacrine (AM), proflavine(PF) and ethidium bromide (EB). These MIC results were confirmed by spectrophotometric measurements of cultures growing in nutrient broth (NB) and by following viability in NB+10 μg/ml AM. The mec^+ count remained constant for 5hr, whereas the mec^- strain grew with a mean generation time of 80 min. When thymineless mutants were obtained by trimethoprim selection, the mec^+thy^- strain underwent classic "thymineless death" showing 0.22% survivors after 5 hr in the absence of thymine, whereas 38% of the mec^-thy^- strain were still viable after the same period.

Strain 1100 mec^+ had been mutagenised with ethylmethane sulphonate in order to obtain the mec^-derviative, and the mec^- allele was therefore transferred into an unmutagenised background to check that the resistance traits were indeed due to absence of 5-MeC. This was achieved by transduction of E.coli strain J6-2 his^-mec^+ with phage P1-lysates of strain 1100 mec^-his^+, and selecting for revertants to his^+. The mec gene is close to the his operon and it was expected that some of the J6-2 his^+ derivatives would also be mec^-. Since the chromosomal mec^+ gene product protects against the EcoRII restriction nuclease, a spot test was devised whereby the efficiency of plating (eop) of phage λvir grown on 169 separate J6-2 his^+ isolates was compared on $RN3^+$ and $RN3^-$ strains. Using this criterion, 16 out of 169 his^+ isolates tested were found to be mec^-, giving a cotransduction frequency of 9.5% and a his-mec distance of 1.1 min. This agrees well with the value of 1 min quoted in the latest E.coli linkage map (Bachmann, et al., 1976). None of the 16 his^+mec^- transductants showed any significant increase in MIC to AC, PF or EB and it is concluded that resistance to these intercalating drugs has not been cotransduced with the mec^- allele.

When the mec^- mutation was introduced into a thy^- derivative of strain J6-2, a series of 8 $mec^-his^+thy^-$mutants was obtained, two of which showed resistance to thymineless death (30% survival after 5 hr thymine starvation compared with 0.027% for the J6-2 mec^+thy^- strain), whilst the others were as sensitive to thymine deprivation as the parent. This could suggest that another gene, closely linked to the mec^- locus, is responsible for resistance to thymineless death, but since none of 50 his^+mec^+ transductants were found to be resistant to thymine starvation, we conclude that the mec^- allele is indeed responsible. It is more likely that those isolates which do not protect against EcoRII restriction (i.e. phenotypically mec^-), but which remain sensitive to thymineless death are stringent with respect to lack of hspII modification activity, but "leaky" in the mec gene function that confers wild type (mec^+) sensitivity to thymine starvation.

Bachmann, B.J., Low, K.B. & Taylor, A.L. (1976). Bact. Rev., 40, 116-167.
Hattman, S., Schlagman, S. & Cousens, L. (1973). J. Bact. 115, 1103-1107.
Hirota, Y. & Iijima, T. (1957). Nature (Lond.) 180, 655-656.
Vanyushin, B.F., Belozersky, A.N. et al. (1965). Nature (Lond.), 218, 1066-1067.