Appendix
APPENDIX

PROPERTIES AND GENOTYPES OF THE TESTER STRAINS OF SALMONELLA TYPHIMURIUM

In addition to the histidine mutation, the standard tester strains contain other mutations that greatly increase the sensitivity in detecting the mutagens. The rfa mutation cause partial loss of the lipopolysaccharide barrier that coats the surface of the bacteria, thus increasing the permeability of large molecules such as benzo(a)pyrene, that do not penetrate the normal cell wall. The other mutation, (uvr B mutation) involves the deletion of a gene coding for the DNA excision repair system, resulting in greatly increased sensitivity in detecting many mutagens (Ames, 1971).

TA 98 has been developed by transferring a resistance transfer factor (R factor) to the standard tester strains TA 1538 respectively. The new strain (TA 98) are extremely sensitive in detecting a number of mutagens and are recommended for use in general mutagenesis testing (Maron and Ames, 1983).

Maintenance of tester strains

The strains were inoculated into nutrient broth (8 g nutrient broth, 5 g NaCl, 1000 ml distilled water) and allowed to grow at 37°C. The genotypes of the tester strains were confirmed as described below and the culture streaked on nutrient agar plates (Master plates).

Confirming genotypes of tester strains

Histidine requirements

The Histidine character of the tester strains was confirmed by demonstrating the histidine requirements for growth on selective agar plates (Biotin is also required by all the standard tester strains because of the uvr B deletion which extends through the biogene).

Each plate (with 0.1 ml of 0.5 mM biotin and with or without 0.1 ml of 0.1M Histidine) was streaked with the strains, incubated overnight at 37°C and examined for growth. The histidine requirement was shown by growth observed only in His/bio plate but not in the control plate with Biotin alone.
rfa mutation

The presence for rfa mutation was checked by testing the permeability of large molecules. Crystal violet was used for this purpose. Sterile filter paper disc, onto which 10 μl of crystal violet solution (1 mg/ml) has been delivered, was carefully placed on the solidified top agar to which the bacterial culture has been added. After overnight incubation at 37°C, a clear zone of inhibition was observed around the disc, indicating the presence of rfa mutation, permitting large molecules like crystal violet to enter and kill the bacteria.

uvr B mutation

The uvr B mutation is quite stable and can be confirmed by demonstrating uv sensitivity in strains that contain the mutation. For this, the cultures were streaked on the nutrient agar plate. The plates were partially covered (so that half of each streak was covered) with a piece of cardboard. The plates were then irradiated with a germicidal UV lamp at a distance of 33-35 cm, for 8 seconds and then incubated overnight at 37°C after which time it was observed that the bacteria grow only on the side that was not exposed to UV.

R. Factor

The presence of R. factor should be tested routinely by the presence of ampicillin resistance, because the plasmas are somewhat unstable can be lost from bacteria. For this, plate-containing ampicillin (25 μg/ml) in the basal agar were prepared and the cultures were streaked on this. After 12.24 hours incubation at 37°C, growth was observed only along the streak made with the R. factor.