Thermosensitive Production of Their Transfer Systems by Group S Plasmids

By V. RODRIGUEZ-LEMOINE,* A. E. JACOB, R. W. HEDGES
AND NAOMI DATTA

*Bacteriology Department, Royal Postgraduate Medical School,
London W12 0HS*

(Received 19 June 1974; revised 15 July 1974)

SUMMARY

Transfer of plasmids of group S is much more efficient at low temperatures (e.g. 22 °C) than at 37 °C. This is due to failure of the donor strain to produce the transfer system during growth at the higher temperature.

INTRODUCTION

A new class of transmissible plasmids has been identified in strains of *Serratia marcescens*. The plasmids constitute the compatibility class S (Hedges, Rodriguez-Lemoine & Datta, 1975).

They were transferred from wild-type *S. marcescens* isolates into strains of *E. coli* K12 by conjugation. There was little or no transfer of these plasmids between strains of *E. coli* K12 when incubation was at 37 °C. When, however, the incubation was at a lower temperature transfer proceeded at a much higher rate. We describe the identification of the temperature-sensitive step in the mating process.

METHODS

Bacteria. Both strains used in these experiments were nutritionally distinguishable derivatives of *E. coli* K12. They were J53 (F⁻, *pro*, *met*) and J62.2 (F⁻, *pro*, *his*, *trp*, *lac*, *rif*).

Plasmids. R477 is an R factor of group S which determines resistance to streptomycin, tetracycline, chloramphenicol, kanamycin and sulphonamides. It and other S plasmids were described by Hedges *et al.* (1975).

Temperature shift experiments. The donor culture, J62.2(R477), was grown overnight in nutrient broth at 37 °C. It was diluted, usually 1:100, in nutrient broth and incubated at the desired temperature for at least 2 h. Cultures were diluted with fresh broth at appropriate intervals in order to maintain the cell concentration at approximately 10⁸ cells/ml, as measured by a Celloscope 302 particle counter (Lars Ljungberg and Co., Stockholm, Sweden).

After preliminary experiments, which showed that the conditions under which the recipient culture was grown before mating had little effect on the efficiency of transfer, recipient cultures were routinely grown to a concentration of approximately 10⁹ cells/ml at the temperature to be used during conjugation.

Mating mixtures were set up by mixing 4.5 ml of the recipient culture with 0.5 ml of the donor culture and adding to this mixture 5 ml of fresh, pre-warmed broth. Mating mixtures

* Present address: Microbiology Department, University College, London W.C. 1.

Fig. 1

Fig. 1. The influence of the temperature of mating mixtures on transfer of R477. The donor culture, J62-2(R477), was grown unshaken at 22 °C. ●, Selection for tetracycline resistance; ○, selection for kanamycin resistance.

Fig. 2

Fig. 2. The influence of the temperature at which the donor culture was incubated before mating. All mating mixtures were incubated at 22 °C. Donor cultures were adjusted to uniform density before mating. For symbols, see Fig. 1.

were incubated at an appropriate temperature for 1 h, after which suitable dilutions were spread on selective agar plates.

Stability of inheritance. The stability of S plasmids at various temperatures was tested as described by Coetzee, Datta & Hedges (1972).

RESULTS

Temperature shift experiments

R477 was the representative S plasmid used in these experiments, which showed that it was the temperature of incubation of the donor culture before mating which determined the efficiency of transfer. If the donor was preincubated at 22 °C the mating proceeded efficiently; the temperature at which the mating mixture was incubated was of little significance (Fig. 1).

Figure 2 shows the effect of preincubation of the donor cultures at various temperatures before mating at 22 °C. Transfer efficiency was highest (4×10^{-3} /donor cell) from the culture incubated at 22 °C. The frequency of transfer from the culture incubated at 37 °C was about 5000-fold less (7×10^{-7} /donor cell).

Fig. 3

Fig. 4

Fig. 3. Acquisition of mating ability at low temperature. The donor culture was grown at 37 °C, and diluted into broth at 22 °C at time 0. At intervals, matings were made at 22 °C. For symbols, see Fig. 1.

Fig. 4. Loss of mating ability at high temperature. The donor culture was grown at 22 °C, and diluted into prewarmed broth at 37 °C at time 0. At intervals, matings were made at 22 °C. For symbols, see Fig. 1.

Figure 3 shows the regeneration of conjugative ability when a donor culture, in log phase, was transferred from 37 to 22 °C. Complete recovery of activity took place within 4 h.

Figure 4 shows the kinetics of loss of conjugative ability when a donor culture in log phase was transferred from 22 to 37 °C. Within 1 h, transfer ability fell approximately 1000-fold.

S plasmids from different sources and with different resistance patterns, R478, R826 and R829 (Hedges *et al.* 1975), were all transferred more efficiently between strains of *E. coli* K12 when the donor cultures were grown at low temperature.

Stability of S plasmids during vegetative growth

The inheritance of plasmids of group S was stable at both 30 and 43 °C.

DISCUSSION

The temperature dependence of plasmid transfer has been previously studied with matings mediated by the F factor and by Rts1. F matings are efficient over a considerable range of temperature (Wood, 1968). Conjugation takes place with high efficiency at 37 °C and markedly less efficiently at 22 °C. Transfer of Rts1 occurs with very low efficiency at 37 °C and is

much more effective at 30 °C or below. This low efficiency of transfer at high temperature is associated with a failure to replicate at such temperatures (Terawaki, Takayasu & Akiba, 1967; Coetzee *et al.* 1972). S plasmids, however, are able to replicate efficiently in *E. coli* K12 growing at high temperatures. Also, Rts1⁺ cultures of *E. coli* grow poorly at 37 °C or above (Terawaki, Kakizawa, Takayasu & Yoshikawa, 1968; Di Joseph, Bayer & Kaji, 1973). Strains carrying S plasmids showed no such abnormalities of growth. As will be discussed, the inefficient transfer of S plasmids at high temperatures results from a failure to synthesize a transfer system rather than a failure of DNA replication. Thus, the mechanisms underlying the thermolability of plasmid transfer are quite different.

The results of the temperature-shift experiments show that a prolonged period of incubation at low temperature is required if a strain of *E. coli* carrying an S plasmid is to acquire the ability to transfer the plasmid. The ability can be expressed at high or low temperature, but if a culture capable of transfer is incubated at 37 °C the ability is progressively lost.

We conclude that at low temperature, the plasmid is able to determine the synthesis of a transfer system that is capable of functioning at 22 or 37 °C, but that at the latter temperature, *de novo* synthesis of one or more components of this system is inhibited.

Since S plasmids seem to be adapted to transfer at temperatures lower than mammalian body temperature and their only known natural host (*S. marcescens*) is often found in soil and water, it is natural to postulate that the S plasmids evolved in *Serratia* populations in environments where low temperatures are usual.

The fact that all the *Serratia* R factors came from strains isolated from human infections (Hedges *et al.* 1975) may imply that genes for antibiotic resistance of advantage in the hospital environment have become associated with plasmids evolved in response to different evolutionary pressures.

V.R.-L. was supported by the Consejo de Desarrollo, Científico y Humanístico, Universidad Central de Venezuela. N.D. was in receipt of a grant from the Medical Research Council.

REFERENCES

- COETZEE, J. N., DATTA, N. & HEDGES, R. W. (1972). R factors from *Proteus rettgeri*. *Journal of General Microbiology* **72**, 543–552.
- DI JOSEPH, C. G., BAYER, M. E. & KAJI, A. (1973). Host cell growth in the presence of the thermosensitive drug resistance factor, Rts 1. *Journal of Bacteriology* **115**, 399–410.
- HEDGES, R. W., RODRIGUEZ-LEMOINE, V. & DATTA, N. (1975). R factors from *Serratia marcescens*. *Journal of General Microbiology* **86**, 88–92.
- TERAWAKI, Y., KAKIZAWA, Y., TAKAYASU, H. & YOSHIKAWA, M. (1968). Temperature sensitivity of cell growth in *Escherichia coli* associated with the temperature sensitive R(KM) factor. *Nature, London* **219**, 284–285.
- TERAWAKI, Y., TAKAYASU, H. & AKIBA, T. (1967). Thermosensitive replication of a kanamycin resistance factor. *Journal of Bacteriology* **94**, 687–690.
- WOOD, T. H. (1968). Effects of temperature, agitation and donor strain on chromosome transfer in *Escherichia coli* K12. *Journal of Bacteriology* **96**, 2077–2084.