

---

## Stimulation of Recombination in *Escherichia coli* K12 by Cations Incorporated into Various Basal Media<sup>1</sup>

JOHN H. LANCASTER and BARBARA S. GALLUP,

University of Oklahoma, Norman

Conjugation between mating types in *Escherichia coli* K12 occurs in four stages (Jacob and Wollman, 1961). These include random collision of cells, formation of stable mating pairs, transfer of chromosome from donor to recipient cells, and integration of donor chromosome fragments into the recipient genome.

Curtiss, Mays, and Stallions (1967) studied the requirements for macromolecular synthesis and energy metabolism during conjugation. Their results indicate no energy requirement for formation of mating pairs, although energy metabolism is required by both donor and recipient cells for chromosome transfer. Protein synthesis is required in the donor cells prior to chromosome transfer. This may be due to the necessity for production of enzymes involved in the initiation of DNA synthesis, or it may involve synthesis of a "conjugation tube" connecting the two mating cells (Anderson, Wollman, and Jacob, 1957).

Although these studies and others (i.e., Fisher, 1957 a, b) established a requirement for energy metabolism in conjugation, relatively little is known about the requirements for specific metabolites. In this report, we will present results of a study designed to ascertain the involvement, if any, of specific cations in conjugation between mating types of *E. coli* K12.

### MATERIALS AND METHODS

**Cultures**—The cultures used throughout this study were Hfr G6 *his* and F 464 *ileu*. Both are derivatives of *E. coli* K12 and were obtained from Dr. E. P. Goldschmidt, Department of Biology, University of Houston, Texas.

**Culture media and preparation of cells for conjugation**—Both donor and recipient cells were grown to cell densities of approximately  $2 \times 10^8$

---

<sup>1</sup>This work was supported in part by funds provided by The University of Oklahoma Faculty Research Fund.

cells per ml in Difco-Penassay broth (Detroit Industrial Fermentations Company). These cells were harvested by centrifugation and washed twice in sterile deionized water. Hfr G6 cells were resuspended to a cell density of  $1 \times 10^8$  cells per ml in deionized water. The F<sup>-</sup> cells were resuspended to the same cell density in the mating medium.

Deionized water was prepared by passing distilled water through a standard Barnstead demineralizer cartridge.

*Vogel's minimal salts*—Glucose agar medium (Vogel and Bonner, 1956) was used for detection of recombinants.

All the basal media employed in this study were prepared with 0.1 M sodium phosphate buffer (pH 7.0) in deionized water. The individual media contained the following additional ingredients: Basal Medium I - 0.2% glucose; Basal Medium II - 0.2% glucose and 0.2% aspartic acid; Basal Medium III - 0.2% glucose and 0.2% citric acid.

*Conjugation experiments*—Conjugation was carried out in the basal media and basal media supplemented with various cations. Hfr G6 and F<sup>-</sup>464 cells were mixed in the mating media in 1:10 ratio. After one hour's mating, aliquots were removed and plated on Vogel's medium for assay of recombinants.

## RESULTS

The ability of various cations to stimulate recombination in deionized water is shown in Table I. With the exception of potassium, all ions were used at a concentration of  $10^{-4}$  M. Potassium was used in  $10^{-3}$  M amounts. These concentrations were found to give optimum stimulation of recombination, although the level of stimulation by magnesium, ferrous iron, and manganese was low. Only calcium and potassium showed marked stimulation of recombination under these conditions. The chloride salts were used in all experiments.

A comparison of recombination obtained in the various basal media is given in Table II. Each of the basal media support recombination better than does deionized water. Those media containing glucose and aspartic or citric acids are much better than the medium containing glucose alone. This is in agreement with earlier observations reported by Fisher (1957 b).

Table III summarizes the effects of cations on recombination levels obtained with each of the basal media. Relatively little effect is seen for any of the cations in Basal Medium I, although some stimulation occurs in the presence of calcium, magnesium, and potassium.

In Basal Medium II, very marked stimulation of recombination is seen in the presence of potassium. Some stimulation is observed with magnesium, while the other ions were ineffective. A similar, but less pronounced, potassium effect is seen with Basal Medium III, which contains citric acid. In this medium, however, incorporation of many of the cations results in a decrease in the number of recombinants compared with those obtained in the basal medium alone.

None of these observed differences can be attributed to variations in viability of the cells in the mating mixtures, since viability controls showed no significant differences in survival in the various media. Approximately 20-30% of the cells died in each case.

## DISCUSSION

The involvement of cations in conjugation has been studied by several laboratories with apparently conflicting results. Kirchner and Eiserstark (1957) reported an increase in recombination in the absence of

divalent cations. Czerwinska (1964) found that addition of potassium to nutrient broth which had been depleted of cations by passage through an ion exchange resin would increase recombination.

The results presented here generally support the observations of Czerwinska, although potassium stimulation appears to depend on the

TABLE I. STIMULATION OF RECOMBINATION BY CATIONS SUSPENDED IN DE-IONIZED WATER.

Cation	Optimum Concentration (M)	Ratio of Recombination Obtained in Cation Media to Recombination in Deionized Water*
Ca <sup>++</sup>	10 <sup>-6</sup>	30.0
Mg <sup>++</sup>	10 <sup>-6</sup>	1.2
Fe <sup>++</sup>	10 <sup>-6</sup>	1.4
Mn <sup>++</sup>	10 <sup>-6</sup>	1.2
K <sup>+</sup>	10 <sup>-4</sup>	45.0

\*2.5 × 10<sup>7</sup> recombinants per ml were obtained in deionized water.

TABLE II. COMPARISON OF THE NUMBER OF RECOMBINANTS OBTAINED WITH VARIOUS BASAL MEDIA.

Basal Medium	Ratio of Recombination Obtained in Basal Media to Recombination in Deionized Water*
I	9
II	100
III	800

\*5.3 × 10<sup>7</sup> recombinants per ml were obtained in deionized water.

TABLE III. EFFECT OF CATIONS ON RECOMBINATION IN BASAL MEDIA

Cation	Ratio of Recombinants Obtained in Cation-Supplemented Media to Those Obtained in:		
	Basal Medium I*	Basal Medium II**	Basal Medium III***
Ca <sup>++</sup>	1.8	0.9	0.7
Mg <sup>++</sup>	1.8	2.0	0.03
Fe <sup>++</sup>	0.8	0.9	0.3
Mn <sup>++</sup>	0.8	1.0	0.35
K <sup>+</sup>	1.6	67.0	9.0

\*2.2 × 10<sup>6</sup> recombinants per ml were obtained in Basal Medium I.

\*\*2.0 × 10<sup>6</sup> recombinants per ml were obtained in Basal Medium II.

\*\*\*2.0 × 10<sup>6</sup> recombinants per ml were obtained in Basal Medium III.

other components of the mating medium. Calcium and magnesium, both of which are divalent cations, will stimulate recombination under some circumstances. Calcium is most stimulatory when incorporated into deionized water. Both calcium and magnesium give approximately two-fold stimulation in glucose-phosphate medium.

Stimulation by potassium may be explained in terms of metabolic effects since it is required for protein synthesis (Lubin and Ennis, 1964). Aspartic acid and citric acid are probably active as primers for the Krebs cycle (Fisher, 1957 a).

The depression of recombination in the presence of divalent cations and citric acid may indicate an interaction between the two which results in a binding of the cations to the citrate molecules. This may effectively interfere with transport of both the citrate and ions across the cell membrane and prevent priming of the Krebs cycle by citrate and possible activation of other enzyme systems by the cations.

Although the stimulatory effects of cations on recombination observed here can be explained in terms of cellular metabolism, this does not eliminate from consideration the possibility that cations may also be involved in surface interactions occurring during pair formation. Studies are currently under way to attempt to separate these two possible functions.

#### SUMMARY

The effect of cations on recombination in various mating media has been studied. Potassium is most striking in its stimulatory effects. Calcium and magnesium are stimulatory under some conditions. Ferrous iron and manganese show no stimulatory effect under any of the mating conditions employed.

#### LITERATURE CITED

- Anderson, T. F., E. L. Wollman, and F. Jacob. 1957. Sur les processus de conjugaison et de recombinaison chez *E. coli*. III. Aspects morphologiques en microscopie electronique. *Ann. Inst. Pasteur.* 93:450-455.
- Curtiss, Roy III, J. A. Mays, and D. R. Stallions. 1967. Macromolecular synthesis and energy metabolism during conjugation in Hfr  $\times$  F<sup>-</sup> matings of *Escherichia coli* K-12. *Bacteriol. Proc.* p. 55.
- Czerwinska, K. 1964. The environmental control of conjugation in *Escherichia coli* K-12. 1. The effect of pH, osmotic pressure, surface tension and cations. *Acta Microbiol. Pol.* 13:3-12.
- Fisher, K. W. 1957a. The role of the Krebs cycle in conjugation in *Escherichia coli* K-12. *J. Gen. Microbiol.* 16:120-135.
- Fisher, K. W. 1957b. The nature of the endergonic process in conjugation in *Escherichia coli* K-12. *J. Gen. Microbiol.* 16:136-145.
- Jacob, F., and E. Wollman. 1961. *Sexuality and the Genetics of Bacteria*. Academic Press, New York.
- Kirchner, C., and A. Eisenstark. 1957. The importance of divalent cations to genetic exchange in *Escherichia coli* K-12 recombination and *Salmonella typhimurium* transduction. *Bacteriol. Proc.* p. 50.
- Lubin, M., and H. L. Ennis. 1964. The role of intracellular potassium on protein synthesis. *Biochim. Biophys. Acta* 80:614-631.
- Vogel, H. V., and D. M. Bonner. 1956. Acetyl ornithinase of *Escherichia coli*: Partial purification and some properties. *J. Biol. Chem.* 218:97-106.