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# Factors Affecting Transformation of *Micrococcus lysodeikticus*

WESLEY E. KLOOS

*Department of Genetics, North Carolina State University, Raleigh, North Carolina 27607*

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Relatively high transfo mation frequencies were obtained with *Micrococcus lysodeikticus* cells, previou"sly grown in defined broth, with a tris(hydroxymethyl)-aminomethane-glutamate buffer containing $Mg^{++}$, $Ca^{++}$, $Ba^{++}$, or $Sr^{++}$.

Recent studies have demonstrated that certain strains of *Micrococcus lysodeikticus* readily undergo transformation with an adenine marker (5; W. E. Kloos, Bacteriol. Proc., p. 55, 1968) and with several histidine and tryptophan markers (W. E. Kloos and N. E. Rose, *unpublished data*) by use of an agar plate technique. However, plate transformation, although sensitive and reproducible, required long incubation periods for the expression of prototrophic recombinants and was associated with a stimulated recipient background. Some limited success has been obtained with transformation in complex liquid media (5, 8, 9). Nevertheless, in these preliminary studies, transformation frequencies were very low and the transformation medium supported growth of recombinants. The present investigation was undertaken to develop an efficient transformation system in a liquid medium that would be more amenable to analysis and could be compared with transformation systems in other bacteria.

Experiments were performed with purified deoxyribonucleic acid (DNA) isolated from strain ISU (*ade*$^+$*-1*). The auxotrophs ISU (*ade*) and ISU (*ade*$^+$*-1 trp-16*) served as recipients to test the transfer of an adenine and tryptophan marker, respectively. Unless indicated otherwise, transformation was accomplished by the following procedure. The recipient strain was grown for 24 hr at 34 C in a defined liquid medium (*p*H 7.3) having the following composition: $K_2HPO_4$, 2 g; $NH_4Cl$, 1 g; $MgSO_4 \cdot 7H_2O$, 70 mg; $FeSO_4 \cdot 7H_2O$, 1 mg; $MnCl_2 \cdot 4H_2O$, 0.5 mg; glucose, 7 g (added after sterilization); biotin, 10 μg; monosodium glutamate, 10 g; L-arginine, 656 mg; L-phenylalanine, 400 mg; L-tyrosine, 500 mg; L-proline, 900 mg; L-isoleucine, 500 mg; L-cysteine, 480 mg; L-methionine, 270 mg; L-tryptophan, 15 mg (for recipient *ade*$^+$*-1 trp-16*), or adenosine, 10 mg (for recipient *ade*); deionized water, 1,000 ml. Cells were washed once in tris-(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.0, 0.05 M) and were suspended in 1.0 ml of Tris buffer containing 0.5% monosodium glutamate and $10^{-2}$ M $MgSO_4$. DNA from the donor strain (5 μg in 0.05 ml) was added, and the mixture was shaken for 45 min at 30 C. Exposure to DNA was terminated by the addition of deoxyribonuclease (5 μg/ml; Worthington Biochemical Corp., Freehold, N.J.). Control experiments indicated that transformation is completely inhibited if deoxyribonuclease is added to donor DNA several minutes before the addition of recipient cells. Cells were washed once in saline, and appropriate dilutions were plated on a defined agar medium (5). Prototrophs were scored after incubation for 72 hr at 34 C.

Certain factors known to affect transformation in other genera (1-4, 6, 7, 10-12) were selected to determine optimal conditions for transformation in *M. lysodeikticus*. Results shown in Fig. 1 suggest that recipient competence was higher in a defined medium, supporting a slow growth rate, than in a more nutritional (complex) medium. Competence was maximal during late logarithmic growth. Studies with *Bacillus subtilis* (1), *B. licheniformis* (6), and *Haemophilus influenzae* (10) have also indicated a higher competence when cells were grown in a defined medium.

Transformation occurred in Tris-glutamate buffer only if one of the divalent cations, Mg, Ca, Ba, or Sr, was present (Table 1). The relative efficiency of these ions in promoting transformation was quite similar to that found in *B. subtilis* (12). The monovalent cations Na or K, the divalent cations Mn, Fe, Zn, Cu, or Ni, or the trivalent cation Fe did not permit transformation. Energy sources such as glutamate, acetate, or glucose added to Tris or to Tris plus $MgSO_4$ ($10^{-2}$ M) buffers were without effect. The optimal temperature for transformation was 25 to 30 C. The *p*H optimum was 6.7 to 7.5.

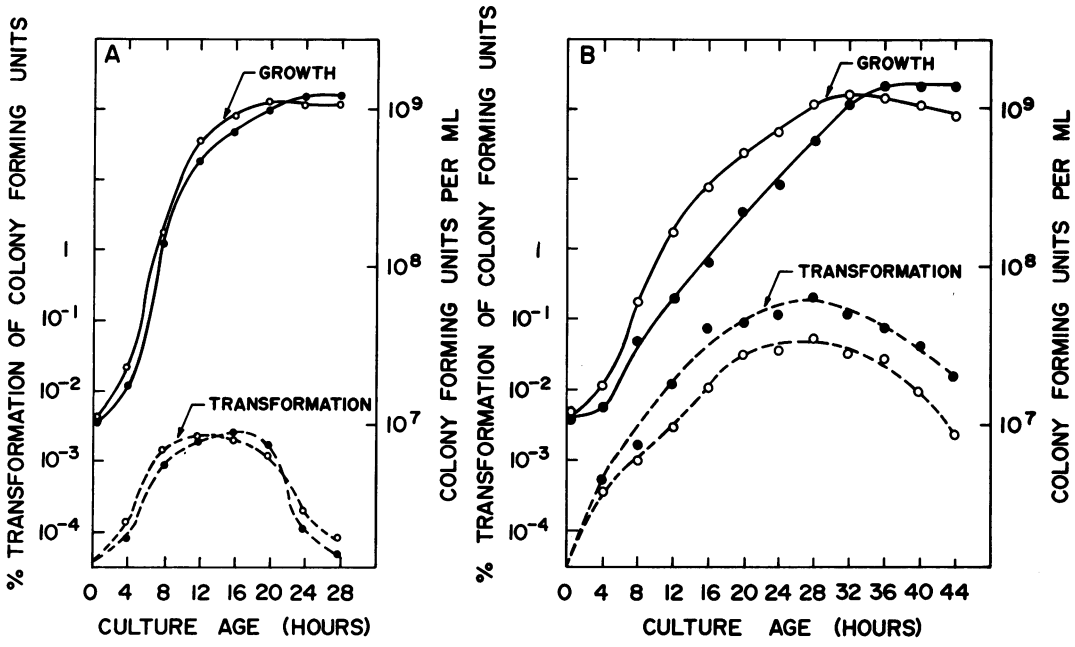


FIG. 1. *Relationship of culture age and recipient competence of cells grown in two different broths. (A) Growth in Nutrient Broth (Difco). (B) Growth in a defined broth. Symbols: ● recipient strain ISU (ade⁺-1 trp-16); ○, recipient strain ISU (ade).*

TABLE 1. *Effect of divalent cations on transformation*

| Ion | Concn[a] | Transformation (% colony-forming units) | |
|---|---|---|---|
| | | *ade*⁺ | *trp*⁺ |
| | M | | |
| None | | <0.0001 | <0.0001 |
| $MgSO_4$ | $10^{-4}$ | 0.0012 | 0.0021 |
| | $10^{-3}$ | 0.0042 | 0.0083 |
| | $5 \times 10^{-3}$ | 0.0370 | 0.0610 |
| | $10^{-2}$ | 0.0500 | 0.0890 |
| $CaCl_2$ | $10^{-4}$ | 0.0012 | 0.0014 |
| | $10^{-3}$ | 0.0086 | 0.0370 |
| | $5 \times 10^{-3}$ | 0.1200 | 0.3800 |
| | $10^{-2}$ | 0.1800 | 0.5700 |
| $BaCl_2$ | $10^{-4}$ | 0.0020 | 0.0020 |
| | $10^{-3}$ | 0.0160 | 0.0510 |
| | $5 \times 10^{-3}$ | 0.1900 | 0.5500 |
| | $10^{-2}$ | 0.2700 | 0.7200 |
| $SrCl_2$ | $10^{-4}$ | 0.0017 | 0.0015 |
| | $10^{-3}$ | 0.1040 | 0.0560 |
| | $5 \times 10^{-3}$ | 0.3100 | 0.5100 |
| | $10^{-2}$ | 0.4600 | 0.7400 |

[a] Concentrations of ions in excess of $10^{-2}$ M were usually inhibitory.

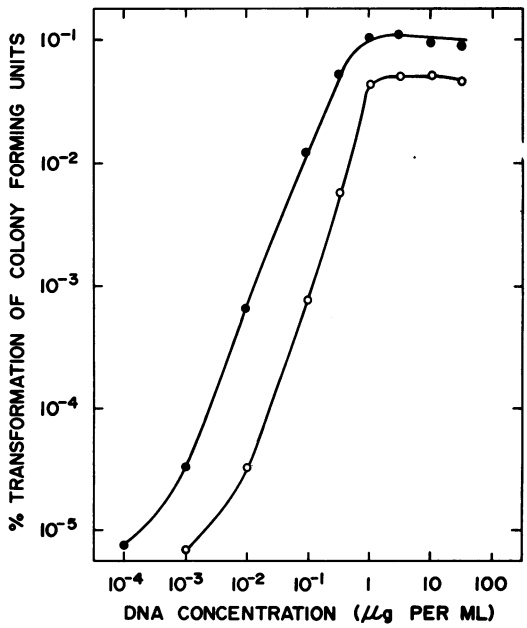


FIG. 2. *Effect of DNA concentration on transformation. Symbols: ●, recipient strain ISU (ade⁺-1 trp-16); ○, recipient strain ISU (ade).*

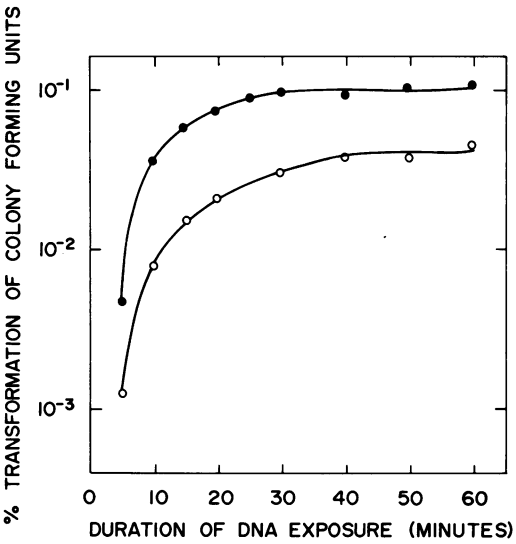


FIG. 3. *Kinetics of transformation. Symbols:* ●, *recipient strain ISU (ade⁺-1 trp-16);* ○, *recipient strain ISU (ade).*

Transformants increased in numbers as the DNA concentration was increased up to a saturation level of about 1 μg/ml (Fig. 2) Transformation reached a maximal value after a 30- to 40-min exposure to DNA (Fig. 3).

All of the factors examined appear to affect transformation in *M. lysodeikticus* in much the same way as has been shown in other genera.

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