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Enhancement and Inhibition of Transformation in *Bacillus subtilis*

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This note describes briefly the effects of several reagents whose presence is stimulatory or inhibitory to transformation in *Bacillus subtilis*.

B. subtilis strains included: SB-5, *his-1 trp-2 ura-1*; SB-19, prototroph; SB-81, *trp-2 his-5*; SB-738, *lys-1*; SB-863, *aro-2 trp-2 tyr-1 his-1 cys-1 leu-1*; SB-871, *ilv-1 met-3*; SB-1006, *aro-2 trp-2 his-2 tyr-1 cys-1 leu-1*; SB-1017, *aro-2 trp-2 tyr-1 cys-1 his-1 lys-1*. (Abbreviations: *his*, histidine; *trp*, tryptophan; *ura*, uracil; *lys*, lysine; *aro*, aromatic amino acids; *tyr*, tyrosine; *cys*, cysteine; *leu*, leucine; *ilv*, isoleucine-valine; *met*, methionine.)

Phleomycin was obtained from Bristol Laboratories, Syracuse, N.Y. Competent cells were prepared by a procedure similar to that described by E. W. Nester, M. Shafer, and J. Lederberg (Genetics **48**:529, 1963). Details of this procedure and of the procedure for the purification of acridine orange will be described elsewhere (C. R. Stewart, *in preparation*). Deoxyribonucleic acid (DNA) was prepared by the Marmur procedure (J. Marmur, J. Mol. Biol. **3**:208, 1961).

Frozen competent cells were thawed, washed with 0.15 M NaCl, 0.01 M tris(hydroxymethyl)aminomethane (Tris), *p*H 7.0, and resuspended at a concentration of approximately 2×10^8/ml in Spizizen's minimal medium (J. Spizizen, Proc. Natl. Acad. Sci. U.S. **44**:1072, 1958), modified or replaced as indicated for each experiment. DNA was added, the tubes were shaken at 30 C for 30 to 45 min, deoxyribonuclease (pancreatic deoxyribonuclease, once crystallized; Worthington Biochemical Corp., Freehold, N.J.) was added to 10 μg/ml, the tubes were shaken for 10 min more, and the cultures were spread on the appropriate plates.

Effect of potassium phosphate. The standard Spizizen's minimal medium contains 0.14 M potassium phosphate. When the phosphate was left out of the solution, the *p*H remained the same (6.9) because of the combination of citrate and ammonium present. (It is necessary to autoclave the ingredients separately at 10 times their normal concentrations, since autoclaving the combined solutions, without the phosphate buffer, produces *p*H changes and markedly lower transformation frequencies.)

TABLE 1. *Effect of potassium phosphate on transformation*[a]

Expt	DNA	*his-1* transformants with T[b] medium		*his-1* transformants with T[b] medium minus potassium phosphate	
		Colonies/ml	Frequency	Colonies/ml	Frequency
1	Linear response range	43×10^3	2.4×10^{-4}	244×10^3	1.3×10^{-3}
2	Saturating concentration	344×10^4	1.8×10^{-2}	397×10^4	2.2×10^{-2}

[a] The transformation mixture for experiment 1 contained 0.07 μg of SB-871 DNA per ml. That for experiment 2 contained 40 μg of SB-19 DNA per ml. The recipient strains were SB-863 for experiment 1 and SB-1017 for experiment 2.

[b] T medium is Spizizen's minimal medium plus 0.02 M $MgCl_2$.

As Table 1 shows, the transformation frequency with DNA concentration in the linear response range was as much as five times as great in this phosphate-free medium as in ordinary Spizizen's medium. When the DNA concentration was at saturating levels, the increase was so slight that it was probably not significant. The latter observation suggests that the phosphate may act as a competitive inhibitor of transformation. Phosphate is also inhibitory in the λ transformation system (A. D. Kaiser, *personal communication*).

Effect of magnesium. The standard Spizizen's

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TABLE 2. *Effect of magnesium on transformation*[a]

Expt	Medium	Transformant colonies/ml	
		Without added Mg^{++}	With 0.02 M $MgCl_2$ added
1	Spizizen's	129×10^1	532×10^1
2	0.01 M Tris, 0.15 M NaCl, *p*H 7.0	90×10^1	374×10^1
3	Spizizen's minus potassium phosphate	141×10^3	297×10^3

[a] The transformation mixtures contained 0.04 μg of SB-871 DNA per ml for experiments 1 and 2, and 0.14 μg of SB-19 DNA per ml for experiment 3. The recipient strains were SB-1006 and SB-863, respectively. The addition of $MgCl_2$ had no effect on the total viable count.

TABLE 3. *Effect of β-mercaptoethanol on transformation*[a]

Mercaptoethanol concn (M)	Transformant colonies/ml		
	Expt 1	Expt 2	Expt 3
0	306×10^3		
4×10^{-6}	328×10^3		
4×10^{-5}	482×10^3		
4×10^{-4}	622×10^3	187×10^2	
8×10^{-4}		298×10^2	221×10^2
16×10^{-4}			214×10^2

[a] The transformation mixture for experiment 1 contained 0.04 μg of SB-871 DNA per ml. That for experiments 2 and 3 contained 0.01 μg of SB-738 DNA per ml. The recipient strain in all cases was SB-81, and the data given are for the *trp-2* marker. Because of the different DNA preparations used, the absolute numbers of colonies are not comparable from one experiment to another. Changes in β-mercaptoethanol concentration did not cause any significant change in total viable count.

minimal medium contains approximately 0.001 M Mg^{++}, and it has been observed (J. Spizizen and C. Anagnostopoulos, Bacteriol. Proc., p. 185, 1960) that the magnesium ion was required for transformation. W. F. Bodmer (J. Mol. Biol. **14**:534, 1965) observed a stimulatory effect of magnesium on DNA uptake during transformation. In our hands, low levels of transformation could be achieved without magnesium, but magnesium increased the transformation frequency, and the optimal concentration was well above that in Spizizen's minimal medium. When 0.02 M $MgCl_2$ was added to the transformation medium, the transformation frequency was increased by two- to fourfold above that obtained in Spizizen's minimal medium alone, with DNA concentrations in the linear response range (Table 2). When the $MgCl_2$ was added to ordinary Spizizen's medium, a white precipitate formed. Since this did not form upon addition of magnesium to Spizizen's medium without potassium phosphate, it was assumed that phosphate is precipitated from Spizizen's medium by magnesium. However, the effect of magnesium is not entirely due to removal of inhibition by phosphate, since, as shown in Table 2, magnesium enhanced transformation even in phosphate-free media.

Effect of β-mercaptoethanol. β-Mercaptoethanol, added to Spizizen's minimal medium, enhanced the transformation frequency by as much as twofold with DNA concentrations in the linear response range. The effect increased with increasing mercaptoethanol concentration up to 8×10^{-4} M. Typical experiments are shown in Table 3. C. Anagnostopoulos and J. Spizizen (J. Bacteriol. **81**:741, 1961) observed that β-mercaptoethanol, added during the second period of growth of competent cells, stimulates the development of competence, apparently as the result of chelation of Cu^{++} ions. These results show that the β-mercaptoethanol also stimulates the transformation process itself.

Effect of acridine orange. Acridine orange forms complexes with DNA (R. F. Steiner and R. F. Beers, Polynucleotides, p. 301, Elsevier Publishing Co., Amsterdam). L. S. Lerman (J. Cellular Comp. Physiol. **64**:Suppl. 1, 1964), in studying the nature of these complexes, has observed that acridines inhibit recombination in bacteriophage; he has also mentioned an inhibitory effect on pneumococcal transformation (L. S. Lerman, Proc. Natl. Acad. Sci. U.S. **49**:94, 1963). Table 4 shows a typical example of the inhibitory effect of acridine orange on *B. subtilis* transformation.

Effect of phleomycin. Phleomycin selectively inhibits DNA synthesis (N. Tanaka, H. Yamaguchi, and H. Umezawa, Biochem. Biophys. Res. Commun. **19**:171, 1963). In transformation of SB-5 by 0.06 μg of SB-19 DNA per ml, the addition of 0.2 μg of phleomycin per ml at the

TABLE 4. *Inhibition of transformation by acridine orange*[a]

Acridine orange concn (M)	*his-1* colonies/ml
0	304×10^2
2×10^{-5}	212×10^2
6×10^{-5}	78×10^1

[a] Acridine orange, at the indicated concentrations, was added to competent SB-5 in Spizizen's minimal medium. SB-19 DNA was added to a concentration of 0.06 μg/ml. The presence or absence of acridine orange made no significant difference in the total viable count.

same time as the addition of DNA caused a 10-fold reduction in transformation frequency, accompanied by about 40% loss of viable count. Similar observations have been made by W. F. Bodmer and S. Grether (*personal communication*).

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