JOURNAL OF BACTERIOLOGY, Jan. 1969, p. 162-165
Vol. 97, No. 1
Copyright © 1969 American Society for Microbiology
Printed in U.S.A.

Heat-sensitive Step in Deoxyribonucleic Acid-mediated Transformation of *Bacillus subtilis*

CHARLOTTE McCARTHY[1] AND EUGENE W. NESTER
Departments of Microbiology and Genetics, University of Washington, Seattle, Washington 98105

Received for publication 23 October 1968

Over 90% of the competent cells in a population of *Bacillus subtilis* lost their competence after being heated to 50 C for 5 min. There was only a slight loss in the number of transformants if the culture was heated for 5 min after the termination of transformation, but 90% of the transformants were lost after 1 hr at 50 C. The population as a whole grew at a slightly faster rate at 50 C than at 32 C. We postulate that a heat-labile factor is required for the uptake or retention (or both) of deoxyribonucleic acid (DNA) in the cell, since uptake of ^{32}P-DNA into a deoxyribonuclease-resistant form was inversely proportional to the time of exposure to heat. Cells that had lost competence after being heated did not regain their competence for at least several hours, although other cells in the population became competent. These data suggest that the heat-labile factor required for competence is synthesized only once during the period that a cell remains competent.

Competent cells of *Bacillus subtilis*, those capable of taking up, integrating, and expressing exogenously added deoxyribonucleic acid (DNA), differ from noncompetent cells in several respects. About half of the competent cells do not engage in DNA synthesis, and the remainder synthesize DNA at one-half the rate of the noncompetent cells of the culture (5); newly transformed cells which have been shown to be competent (7) synthesize protein, but synthesize only negligible amounts of stable ribonucleic acid (5). The competent cells are resistant to penicillin killing, presumably because they are not synthesizing cell walls (7, 8). This biosynthetic pattern is reminiscent of sporulating cells (2), and, indeed, Young and Spizizen (11) suggested that competent cells are in a presporulation stage. In an attempt to gain additional insight into the possible relationship between sporulation and competence, we investigated the effect of elevated temperatures on competent and newly transformed cells. The results of these experiments show a distinct effect of heating on the competent cell, with no discernible effect on the noncompetent fraction of the population.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains employed are listed in Table 1.

Chemicals. Deoxyribonuclease I was purchased from Worthington Biochemical Corp., Freehold, N.J. Carrier-free ^{32}P in water was obtained from New England Nuclear Corp., Boston, Mass.

DNA. The unlabeled DNA used in the transformations was isolated by the method of Marmur (6) with the omission of ribonuclease treatment and isopropanol precipitation. ^{32}P-DNA was prepared by growing a wild-type strain (WB 746) in the medium and under the conditions described by Young and Spizizen (11). The DNA was isolated by the procedure of Marmur (6), except that the preparation was not precipitated with isopropanol. The specific activity of the preparation was 4.9×10^4 counts per min per μg of DNA. Since no special effort was made to remove teichoic acid (10), some of these counts may have been in this material.

Transformation procedure. The details of the transformation procedure have been described (5). Approximately 5% of the cells are rendered competent by this procedure (8).

RESULTS

Effect of elevated temperature on competent cells. A competent culture of *B. subtilis* 168 (*trp-2*) was shaken at 50 C for various periods of time, cooled to 32 C, and then transformed with SB 491 Trp$^+$ DNA to determine the number of competent cells. The results in Fig. 1 indicate that after 5 min at 50 C more than 90% of the competent cells were no longer competent. This loss was exponential with time and, in this experiment, only 0.01% competent cells remained after 15 min.

Effect of treatment at 50 C on transformed

[1] Present address: Division of Research, National Jewish Hospital, Denver, Colo. 80206.

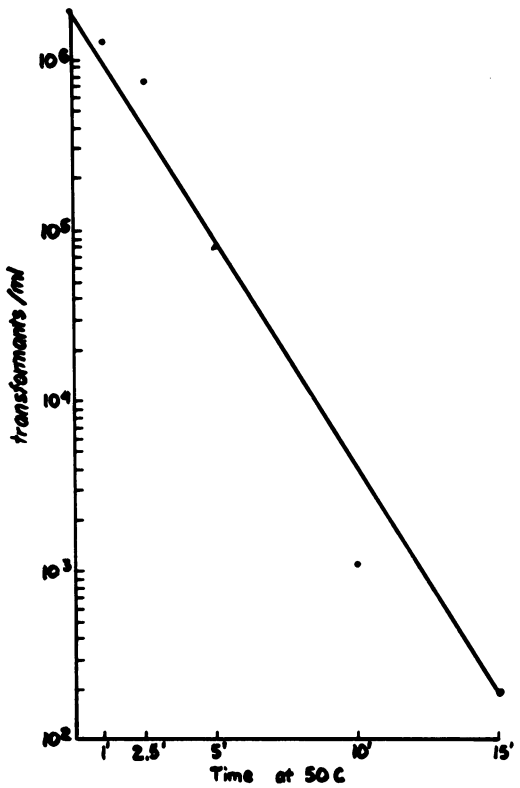

FIG. 1. *Effect of heating at 50 C on competency. A competent culture of B. subtilis 168 was distributed, 0.9 ml/tube, into each of six tubes, and was aerated for the time indicated at 50 C. Saturating SB 491 DNA (0.1 ml) was added, and the culture was aerated at 32 C for 30 min. Deoxyribonuclease (20 μg) was added for 5 min. Competency was determined by the number of Trp+ transformants per milliliter.*

cells. Heating did not kill the competent cells at this rapid rate. Newly transformed cells, which are still competent (7), were readily recovered after heating for 15 min at 50 C. There was, however, about a 90% loss after 60 min of incubation at 50 C; no further loss was evident after 120 min (Fig. 2). In experiments involving the transformation of other mutants, the loss of transformants was even less. The total population grew slightly better at 50 C than at 32 C, as illustrated in Fig. 2.

Uptake of DNA by heated cells. In an effort to determine the step in the transformation process susceptible to heat, ^{32}P-labeled DNA was added to competent cultures which had been heated for various times. After addition of deoxyribonuclease, the cultures were assayed for transformants and for the uptake of ^{32}P into a deoxyribonuclease-insensitive form. The results indicated a close correlation between the loss of transformants and the loss of the ability to take up radioactive DNA (Fig. 3).

Recovery of competency in cultures treated at 50 C. The above experiments suggest that incubation at 50 C destroys a heat-labile factor required for the uptake or maintenance (or both) of DNA in a deoxyribonuclease-insensitive form. To determine whether this heat-labile factor, once lost, could be regained, a culture of strain SB1 (*trp-2 hisA1*, unlinked) was transformed to Trp+ with strain SB 197 (*hisA1*) DNA, heated at 50 C for 10 min, and incubated at 32 C. The competence of these same cells was then assayed by transforming with Trp− His+ DNA (strain 168) at periodic intervals and scoring for double transformants (Trp+ His+). The culture as a whole regained competence (Table 2), but the cells which had already been transformed

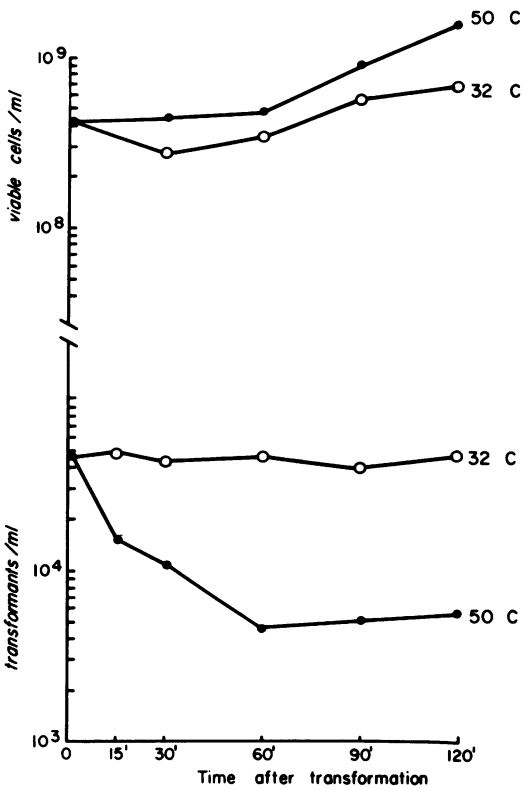

FIG. 2. *Comparison of the effect of 50 C and 32 C on viability of transformants and the total population. A competent culture of B. subtilis 168 was transformed by 5-min exposure to SB 491 DNA followed by 1 min of deoxyribonuclease treatment. The culture was divided into two portions; one was aerated at 32 C and one at 50 C. Plate counts were made at the times indicated after deoxyribonuclease addition.*

were unable to regenerate competence within 180 min. Only a negligible number of double transformants were obtained from the culture treated at 50 C (Table 2). If the culture was not heated, double transformants were readily detected. Similar results were obtained with SB 25 (*trp-2 hisB2*, linked) as the recipient culture and the appropriate donor *hisB2* DNA.

Heat causes a phenotypic, rather than genotypic, loss of competence. Heated Trp^- His^+ transformants of SB 1 (*trp-2 hisA1*), when isolated and grown under the usual competence regime, were fully capable of being transformed to Trp^+. Hence, the heating did not result in a loss of genetic information specifying competence.

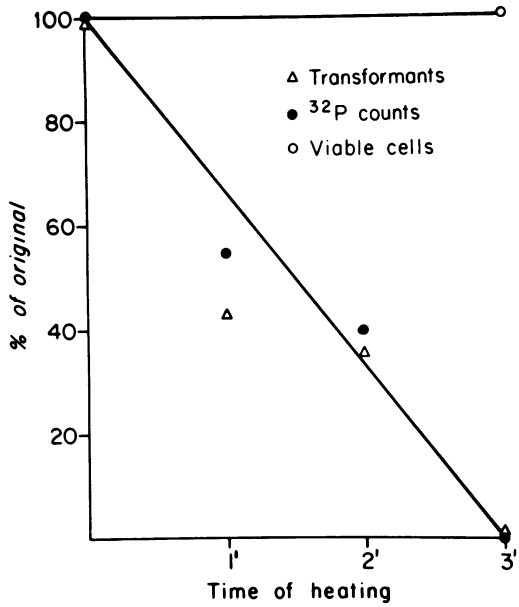

FIG. 3. *Effect of heating at 50 C on DNA uptake and transformation. Four flasks, each containing 10 ml of a competent culture (168), were heated to 50 C for the times indicated. The cultures were then placed at 32 C, ^{32}P-DNA (WB 746) was added (3.4 μg/ml, final concentration; 4.9 × 10^4 counts per min per μg), and incubation was continued for 30 min. Deoxyribonuclease (5 μg/ml) was then added, and incubation was continued for 5 min. The cells were then centrifuged, washed seven times with minimal medium (9), filtered onto a membrane filter (Millipore Corp., Bedford, Mass.) which was washed with 5% trichloroacetic acid, dried, and placed in a vial containing 10 ml of toluene and Liquifluor. The background counts were obtained by adding deoxyribonuclease to an unheated competent culture immediately prior to adding DNA and processing the culture as described above. Counting was done in a Beckman model ^{L}100 spectrometer. The assays for competent and viable cells were done prior to washing the cells. The culture, without being heated, incorporated 4,371 counts/min after the background (600 counts/min) was subtracted. There were 1.2 × 10^6 Trp^+ transformable cells per ml in the unheated culture.*

TABLE 1. *Strains of Bacillus subtilis*

Strain no.	Genotype[a]
168	*trp-2* (try_2^-)
SB 1	*trp-2 hisA1* (try_2^- his_1^-)
SB 25	*trp-2 hisB2* (try_2^- his_2^-)
SB 197	*hisA1* (his_1^-)
SB 491	prototroph
WB 746	168 revertant

[a] Symbols: *trp* and *try* = tryptophan; *his* = histidine; symbols in parentheses are previous designations.

DISCUSSION

These results suggest that there is a heat-labile factor crucial to the uptake or retention (or both) of DNA in a deoxyribonuclease-insensitive state in the cell. The data do not permit a distinction between these two possibilities. This factor is apparently not concerned with normal cell growth, since cell multiplication at 50 C appears to be unaffected. The data show that, once the factor is inactivated, the cell does not replace or repair it for at least several hours. This inability to restore the factor may be a reflection of the restricted biosynthetic capacity of the competent cell (5, 7, 8). A water-soluble polypeptide competency factor has been described (1), but the heat sensitivity of this factor has not been reported.

One could postulate that the rapid loss of competent cells and the slower, yet significant, loss of transformants upon heating at 50 C could have a common basis. Irreversible fixation of donor DNA does not occur immediately upon DNA uptake, since it can be displaced from the recipient cells by exogenously added DNA (4). If heating destroys some factor concerned with retention or the site of retention, the presence of DNA at this site may confer some protection from the heat.

The results in Table 2 clearly indicate that newly competent cells can arise in the population. Such "cycling" of competency in a *B. subtilis* population was not readily discernible in previous experiments (7), probably because the initially competent cells maintain their competence during the interval in which other cells are achieving competence (4, 7). A similar, much shortened, cycling period of competence has been observed in pneumococcus (3).

TABLE 2. *Recovery of competence within transformed cells made incompetent by treatment at 50 C*

Treatment	Time assayed (min after transformation)	Transformants/ml (Trp^+)	Competent cells/ml (His^+)	Competent transformants/ml (Trp^+ His^+)	Total viable cells/ml
Heated[a]	0	7.5×10^4	1.3×10^5	7.8×10^3	9.1×10^7
	10	8.6×10^4	1.7×10^3	1.0×10^1	1.1×10^8
	90	1.1×10^5	1.1×10^3	<5	3.3×10^8
	180	1.6×10^5	3.1×10^4	ca. 2×10^1	6.6×10^8
Not heated[b]	0	8.8×10^4	1.5×10^5	7.0×10^3	9.4×10^7
	10	6.8×10^4	1.4×10^5	9.0×10^3	1.0×10^8
	90	5.3×10^4	1.4×10^5	3.8×10^3	3.0×10^8
	180	2.5×10^4	6.9×10^4	1.7×10^3	4.7×10^8

[a] Cells of SB 1 (*trp-2 hisA1*, unlinked) were heated for 10 min at 50 C after transformation for the Trp^+ marker, with loss of 3 logs of competent cells, and were incubated at 32 C for the remainder of the experiment. Competency was assayed, at the times indicated, by transformation to His^+.

[b] Cells were incubated at 32 C for the entire experiment.

ACKNOWLEDGMENTS

Part of this study was carried out during the tenure of Public Health Service Pre-doctoral Fellowship 5-F1-GM-28,412 awarded to the senior author, and part was supported by National Science Foundation grant GB 5951.

LITERATURE CITED

1. Akrigg, A., S. R. Ayad, and G. R. Barker. 1967. The nature of a competence-inducing factor in *Bacillus subtilis*. Biochem. Biophys. Res. Commun. 28:1062–1067.
2. Halvorson, H. O., J. C. Vary, and W. Steinberg. 1966. Developmental changes during the formation and breaking of the dormant state in bacteria. Ann. Rev. Microbiol. 20:169–188.
3. Hotchkiss, R. 1954. Cyclical behavior in pneumococcal growth and transformability occasioned by environmental changes. Proc. Natl. Acad. Sci. U.S. 40:49–55.
4. Kammen, H. O., R. J. Wojnar, and E. S. Canellakis. 1966. Transformation in *Bacillus subtilis*. II. The development and maintenance of the competent state. Biochem. Biophys. Acta 123:56–65.
5. McCarthy, C., and E. W. Nester. 1967. Macromolecular synthesis in newly transformed cells of *Bacillus subtilis*. J. Bacteriol. 94:131–140.
6. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3:208–218.
7. Nester, E. W. 1964. Penicillin resistance of competent cells in deoxyribonucleic acid transformation of *Bacillus subtilis*. J. Bacteriol. 87:867–875.
8. Nester, E. W., and B. A. D. Stocker. 1963. Biosynthetic latency in early stages of deoxyribonucleic acid transformation in *Bacillus subtilis*. J. Bacteriol. 86:785–796.
9. Spizizen, J. 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. Proc. Natl. Acad. Sci. U.S. 44:1072–1078.
10. Young, F. E., and A. P. Jackson. 1966. Extent and significance of contamination of DNA by teichoic acid in *Bacillus subtilis*. Biochem. Biophys. Res. Commun. 23:490–495.
11. Young, F. E., and J. Spizizen. 1961. Physiological and genetic factors affecting transformation of *Bacillus subtilis*. J. Bacteriol. 81:823–829.