A SUBSTANCE PRODUCED BY COMPETENT BACILLUS CEREUS 569 CELLS THAT AFFECTS TRANSFORMABILITY

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Tomasz and Hotchkiss[1] reported recently that pneumococcal cells became competent when a macromolecular cell product, probably protein in nature, was produced. This "activator" was reported to be transferrable to non-competent cells in the absence of cell contact, thereby conferring upon them the capacity to absorb DNA and become genetically transformed. Evidence that a substance, which we call "competence factor", is produced by cells of Bacillus cereus strain 569 during a transient competent period, will be presented in this paper. Furthermore, absorption of methylene blue by cells, as detected by the method of Jensen and Haas[2] can be correlated with the onset of competence and the production of the "competence factor".

B. cereus strain 569 was made competent by the procedure of Felkner and Goldschmidt (unpublished data). They grew cells for 16 hr in Brain Heart Infusion (BHI) broth after which 0.1 ml was inoculated into 10 ml of M-1 medium, which is Spizizen's minimal salts medium[3]

supplemented with 0.004% tryptophan, 0.05% casein hydrolysate and 10^{-4}M $FeSO_4(NH_4)_2SO_4$. Following 4 hr growth, the cells were washed one time with the minimal salts (MS) of Spizizen's medium and resuspended at an optical density of 0.100 (600 mμ filter) in M-2 medium (M-1 with tryptophan reduced 8-fold and casein hydrolysate reduced 5-fold). The test system involved transforming streptomycin-sensitive cells with DNA from streptomycin-resistant cells. Cells grown in M-1 are non-competent but achieve maximum competence during growth in M-2 within 60 min after inoculation. Competence was lost completely after 120 min; thus, the physiological state of competence was observed to be transient.

Cells growing in M-2 were removed at varying time intervals and reacted with methylene blue. Highly competent cells displayed a greater affinity for the dye than did non-competent cells when washed with MS before adding them to the dye. If, however, distilled water was used to wash the cells before reacting them with methylene blue, cells taken from all stages of growth showed little affinity for the dye and were all non-competent. Multiple washings were necessary since a single washing left a small proportion of the cells still competent. This suggested that some substance(s) responsible for competence was extracted from competent cells by the distilled water. The extracted material, "the competence factor," was concentrated by vacuum distillation, and when it was added back in appropriate concentrations to cells rendered non-competent by washing with distilled water, their ability to become transformed by DNA was restored. Cells washed once with distilled water after growing for 30 min in M-2 yielded the highest number of transformants upon the

addition of exogenous competence factor; cells grown 75 min or longer in M-2 prior to washing responded negatively to the addition of "competence factor" (Fig 1). Even, non-competent cells taken directly from growth in M-1 were rendered transformable by addition of the "factor". These observations suggest that an optimum concentration of "competence factor" is necessary for maximum transformation to occur. This postulate was strengthened by an experiment designed to show a quantitative relationship between cells and "competence factor." For this experiment, increasing concentrations of non-competent cells were added to a series of tubes each containing the same amount of concentrated "competence factor." After 30 minutes at 37 C the cells

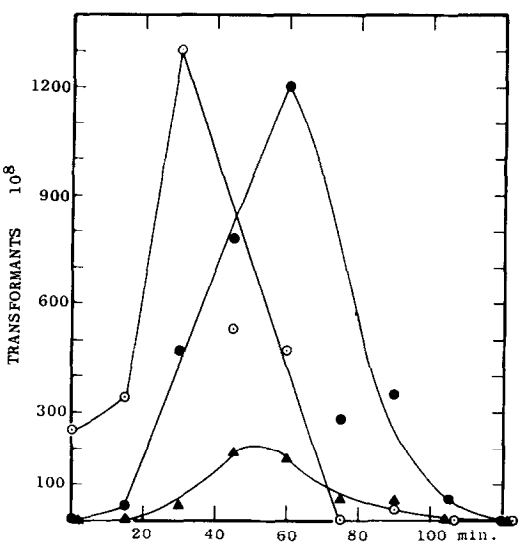

Figure 1. Comparison of the number of B. cereus strain 569 cells transformed by DNA from streptomycin-resistant cells grown for different lengths of time in M-2.

● Cells washed with MS before the addition of DNA.

▲ Cells washed once with distilled water before DNA was added.

O Cells washed with distilled water and "competence factor" added to them before exposure to transforming principle.

TABLE I

Effect of adding increasing concentrations of non-competent cells to a constant concentration of concentrated "competence factor."

OD^{600} of cells used to absorb out activity of "competence factor"	Residual Activity (Transformants/10^8 cells)
0.398	300
0.301	600
0.222	1900
0.155	800
0.097	700
0.046	600
0.000	0
Control cells (non-competent) without factor added.	0

were removed by centrifugation and the various supernatant fluids were assayed for residual "competence factor" by measuring ability to confer competence on non-competent cells. The assay yielded no transformants when the concentration of cells used to absorb out activity was either too high or too low; with intermediate cell densities the residual factor was optimum and maximum transformability occurred.

To determine if any interaction occurred DNA was incubated with "competence factor" for 30 min. Washed non-competent cells were added to this DNA-"competence factor" mixture and aliquots removed at 5 min intervals. These samples were impinged on membrane filters and washed with MS to remove DNA not yet permanently incorporated. As controls,

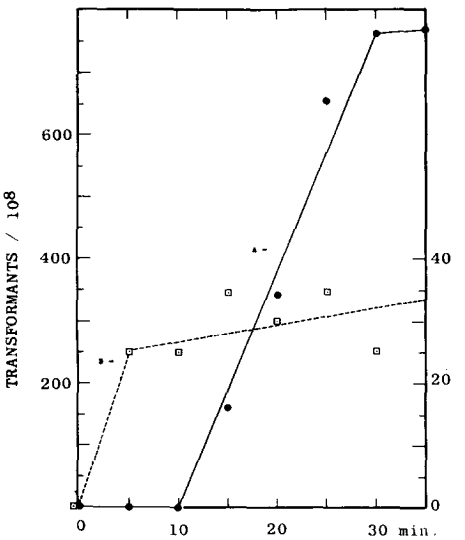

Figure 2. Determination of the interaction of DNA with "competence factor." A-DNA incubated with competent cells, aliquots removed at 5 min intervals and washed with MS. B - DNA - "competence factor" mixture added to non-competent cells, samples removed at 5 min intervals and washed with MS. The number of streptomycin-resistant transformants arising from these samples was used as a measure of the time required to permanently incorporate DNA. Ordinate for A is on left and for B is on the right.

competent and non-competent cells were treated in the same manner with DNA alone. The results revealed the time at which transforming principle could no longer be removed from cells by washing with MS (Fig. 2). Although the transformation frequency was reduced, it appeared that DNA was permanently incorporated much faster when prereacted with "competence factor" than when added directly to competent cells. Transformation did not occur when DNA was added to non-competent cells.

At present, the chemical nature of the "competence factor" is not known. Accumulation begins shortly after the cells are transferred from M-1 to M-2. An optimum concentration is attained by 60 min; upon further incubation it seems likely

that the factor is present in excess, causing the cells to lose their affinity for taking up DNA. The evidence presented here does not exclude the possibility of an activator and an inhibitor being responsible respectively for the cell gaining competence and then losing it after further growth[1]. At present, it seems more likely that in the B. cereus strain 569 system, the key to maximum transformation is having the "factor" present in an appropriate concentration per cell.

REFERENCES

[1] Tomasz, A. and Hotchkiss, R.D., Proc. Natl. Acad. Sci., 51, 480 (1964).
[2] Jensen, R.A. and Haas, F.L., J. Bacteriol 86, 73 (1963).
[3] Spizizen, J., Proc. Natl. Acad. Sci. 44 1072 (1958).