Transformation of Germinated Spores of *Bacillus subtilis* on Agar Plates

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L-Alanine-germinated spores of *Bacillus subtilis* developed a competence on agar plates after 10 h of incubation. Addition of marker amino acid to the plates was required for the transformation.

Spizizen, in his paper first describing the transformation of *Bacillus subtilis*, showed the competence for transformation developed from germinated spores (6). Later, Ephrati-Elizur found high competence of germinated spores in more limited nutritional conditions (3). In either case, nutrients required for growth were present in the inoculum. This report shows that, in development of competence and transformation on agar plate cultures of germinated spores, the presence of required amino acids is necessary.

Spores of *B. subtilis* (Marburg 168, thy trp-2; 5) were prepared in Schaeffer medium as previously described (8). Germination was initiated at 37°C in Spizizen glucose-minimal medium (6) containing 0.1 M L-alanine. Turbidity dropped to the minimum after 60 min. Deoxyribonuclease I (EC 3.1.4.5) added to the DNA-germinated spore mixture had been incubated. Deoxyribonuclease was effective in inhibiting the transformation until the 10th h of incubation (Fig. 3), indicating that germinated spores attain competence after long periods, probably passing through several cell divisions under the limited nutrient conditions.

When vegetative cells were harvested at the log phase of growth, washed repeatedly, and then plated with DNA, the deoxyribonuclease-insensitive stage appeared approximately 10 h after incubation but with lower transformation frequency than found in germinated spores.

With a multiple auxotroph of *B. subtilis* HLL3g derived from strain 168 (ade-6 leu-8 hisA lys-21 thr-5 trp-2 metB5; 7), transformation of germinated spores for each marker occurred on plates supplemented with 1 μg of the amino acid of the transformation marker per ml, 100 μg each of the other required amino acids per ml, and 10 μg of adenine per ml. The deoxyribonuclease-insensitive stage appeared with the same timing as described above. However, the precise order of transformation for each marker that had been determined by
Erickson and Braun with a suspension of germinated spores (4) could not be measured with this system, because different supplements to plates for detection of different markers possibly changed the physiological conditions of cells to be transformed. Consequently, the percent competence could not be determined.

Spores germinated without required amino acids contain unreplicated DNA. This is an ideal system for examining whether or not recombination in genetic transformation requires DNA replication (1, 2). The present results showed that the presence of required amino acids is necessary and seem to indicate a requirement of DNA replication for transformation. However, a possibility remains that the inability to transform germinated spores is due to lack of receptors on the surface, since in another experiment the uptake of $^{14}$C-thymidine-labeled DNA by germinating spores in liquid medium was undetectable.

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LITERATURE CITED

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cient strains of *Bacillus subtilis* by deoxyribonucleate.