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Spizizen J. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. *Proc. Nat. Acad. Sci. US* **44**:1072-8, 1958. [Department of Microbiology, Western Reserve University School of Medicine, Cleveland, OH]

In this paper, published 26 years ago, I demonstrated DNA mediated genetic transformation in *Bacillus subtilis.* As this organism could grow in simple minimal media, it was possible to utilize a variety of auxotrophic markers. This made it possible to investigate the genetic controls of biosynthetic pathways as was being done in *Escherichia coli* using other gene transfer systems [The SCI® indicates that this paper has been cited in over 1,010 publications since 1958.]

John Spizizen
Department of Microbiology
and Immunology
University of Arizona
Health Sciences Center
Tucson, AZ 85724

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"We had previously attempted to transform *Escherichia coli* auxotrophs without success. However, DNA-protein complexes prepared from denatured T2 bacteriophage were found to 'transform' *E. coli* protoplasts,[1] that is, replication and maturation of the phage would occur. These experiments suggested to us that cell wall components were barriers to naked DNA entry. It was at this time that I speculated that *Bacillus subtilis* might be a promising organism, since during stages of outgrowth of germinating spores, cell wall components were minimal or entirely absent.

"Fortunately, Charles Yanofsky was able to provide me with several stable auxotrophic mutants of *B. subtilis* which had been isolated by Burkholder and Giles at Yale University.[2] Transformation of an indole-requiring strain (168) with DNA isolated from a wild strain (W23) was readily achieved. Although germinating spores were initially employed, vegetative cells grown in minimal medium suitably supplemented with a low concentration of casein hydrolysate were found to be highly transformable. Refinements to achieve optimal conditions for transformation were later introduced.[3] These studies demonstrated that specific growth conditions presumably affecting wall synthesis would allow cells to become 'competent' for transformation. The original concept that spore germination would provide the competent state was thus incorrect. Furthermore, other processes besides DNA permeability are now known to be involved in DNA transformation by competent cells. These include a series of coordinated enzymatic steps following DNA attachment, DNA cutting, separation of double strands, penetration of single strands, and pairing with homologous regions of the resident chromosome. The specific enzymes and genetic control of these reactions remain to be identified. B. subtilis strain 168 has the capability of carrying out this coordinated process. It was fortuitous that this strain was used as many if not most strains, including the donor strain W23, could not be made competent for chromosomalDNA transformation.

"Many papers describing modified procedures for transformation have appeared. However, continued reference to the original publication has been made mainly because of the minimal medium used. This simple medium has been employed for transformation in numerous laboratories, as well as for bacterial growth when supplemented with casein hydrolysate and required growth factors. It is ironic that this minor item in the publication is the one quoted most frequently

"Nevertheless, this paper provided impetus for investigations of new areas of procaryotic genetics. These include the genetic control of sporulation, a primitive form of differentiation, and synthesis of extracellular proteins and wall polymers characteristic of gram-positive bacteria.[4]

"I was fortunate to have been stimulated by Yanofsky and Howard Cest to initiate and pursue these studies while at Western Reserve University."

1 **Spizizen J.** Infection of protoplasts by disrupted T2 virus. *Proc Nat. Acad. Sci. US* **43**:694-701. 1957.

2 **Burkholder P R & Giles N H, Jr.** Induced biochemical mutations in Bacillus subtilis. *Amer. J. Bot.* **34**:345-8. 1947. (Cited 105 times since 1955.)

3. **Anagnostopoulos C & Spizizen J.** Requirements for transformation in *Bacillus subtilis. J. Bacteriology* **81**:741-6, 1961. (Cited 615 times.)

4. **Dubnau D A,** ed. *The molecular biology of the bacilli. Volume I:* Bacillus subtilis. New York: Academic Press. 1982. 378 p.