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Satellite Deoxyribonucleic Acid from Bacillus cereus Strain T

Abstract. *DNA isolated from exponentially growing cultures of* Bacillus cereus *T has a single component (density 1.696 g cm^{-3}) in a cesium chloride density gradient whereas DNA isolated from spores shortly after the initiation of germination has two components: a major one (density 1.696 g cm^{-3}) and a satellite (density, 1.725 g cm^{-3}). The DNA of both components is double-stranded. By the first cell division there is no satellite DNA.*

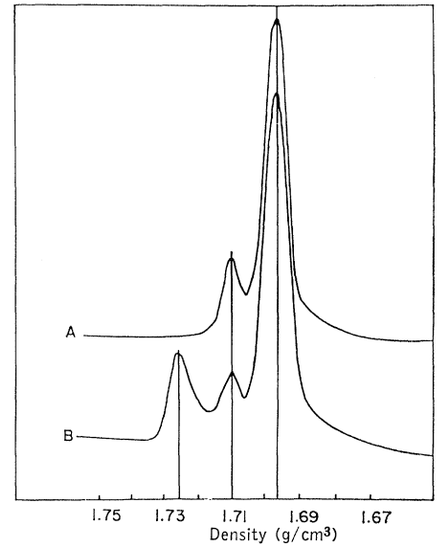
The germination and outgrowth of bacterial spores has been proposed as a useful model system for the study of cellular differentiation (*1*). Evidence has been obtained for changes in the pattern of RNA synthesis (*2*), in classes of ribosomes (*3*), in metabolic activity (*4*), and in structures (*5*). We have examined some of the physiochemical properties of the DNA isolated from spores and outgrowing cells of *Bacillus cereus* T, and have obtained evidence for two DNA components, one of which differs quantitatively and qualitatively from the single component found in logarithmically growing cells of *B. cereus*.

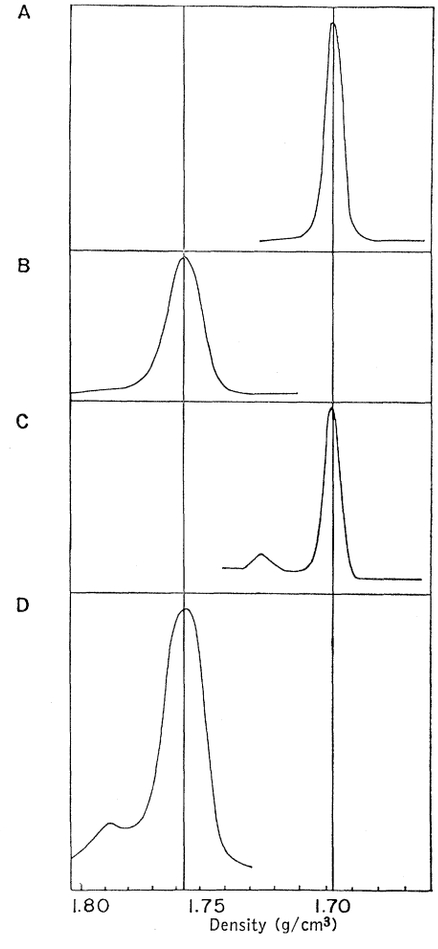
Spores were prepared from cells of *B. cereus* grown in "G" medium (*6*). Germination after heat activation (65°C, 2 to 4 hours) was initiated by the addition of spores to "G" medium containing $10^{-3}M$ L-alanine and $10^{-3}M$ adenosine. Germination was stopped at intervals after initiation by pouring portions of the culture over an equal volume of ice maintained at −20°C. The cells were washed once in SCET buffer [1*M* NaCl, 1 percent cetyltrimethylammonium bromide (weight/volume), $10^{-3}M$ EDTA, and $10^{-3}M$ tris, *p*H 8.0], suspended in the same buffer, and incubated at 37°C overnight with 1 mg of Pronase (Calbiochem) per milliliter. Treatment with Pronase greatly increased the viscosity of the germinated spore suspension, and the spores appeared empty under the phase-contrast microscope. Dormant spores were not appreciably affected. The partially lysed cells were then heated for 4 hours at 65°C to complete lysis and to allow autodigestion of Pronase. Cellular debris was then separated by centrifugation and the supernatant was poured into approximately 4 volumes of 95-percent ethanol. The fibrous precipitate that rose to the top of the ethanol was collected and dissolved in saline-citrate (0.15*M* NaCl + 0.015*M* $Na_3C_6H_5O_7$, *p*H 7.0) according to the procedure of Marmur (*7*). RNA was digested by incubation at 37°C for 30 minutes with 1.0 unit of T_1 ribonuclease and 100 μg of pancreatic ribonuclease per milliliter. Sodium perchlorate was added to a final concentration of 1.0*M* and the incubation mixture was repeatedly deproteinized by a mixture of chloroform and isoamyl alcohol (*7*). The DNA was finally precipitated with ethanol, and the precipitate was dissolved in saline-citrate solution. Such solutions were stored at 4°C over chloroform until used.

Fig. 1 (above right). Buoyant densities in CsCl of DNA extracted from cells in the mid-log phase of growth (*A*) and spores 1 minute after initiation of germination (*B*). *Escherichia coli* DNA (1.71 g cm^{-3}) was used as a reference density marker.

Fig. 2 (bottom right). Buoyant densities in neutral and alkaline CsCl of one and two component DNA from *Bacillus cereus* T.: DNA at neutral *p*H from cells in the mid-log phase of growth (*A*), DNA at *p*H 12 from cells in mid-log phase of growth (*B*), DNA at neutral *p*H from cells shortly after germination (*C*), and DNA at *p*H 12 from cells shortly after germination (*D*).

Analytical methods were essentially those of Meselson, Stahl, and Vinograd (*8*). For analysis in cesium chloride solutions at neutral *p*H, mixtures were prepared by the procedure of Schildkraut, Marmur, and Doty (*9*). When alkaline CsCl solutions were used as solvent, solid CsCl was added to the DNA solution, the density was adjusted





by the addition of $10^{-3}M$ EDTA, and the *p*H was adjusted to approximately 12 by the addition of 1*M* NaOH. The solutions were centrifuged at 44,770 rev/min for 20 hours in the An-D rotor in the Spinco Model E ultracentrifuge with ultraviolet optics. When a saturated aqueous solution of Cs_2SO_4 was used, it was adjusted to the desired density by the addition of the DNA solution and water, and the mixtures were centrifuged at 31,410 rev/min for 40 hours (*10*).

Figure 1 shows densitometric tracings of two typical CsCl gradients. When DNA was extracted from cells in the mid-log phase of growth, only one component was present, which bands in a symmetrical peak at a density of 1.696 g cm^{-3} when *E. coli* DNA is used as a density marker (density, 1.710 g cm^{-3}). However, if the DNA is extracted from spores shortly after the beginning of germination, a second component is evident with a buoyant density of 1.725 g cm^{-3}. Application of the equation of Sueoka, Marmur, and Doty (*11*) indicated that these two components have guanosine plus cytosine contents of 36.7 percent and 66.4 percent respectively.

The material in the satellite peak is (i) insensitive to two kinds of ribonuclease; (ii) insensitive to either pronase or chloroform:isoamyl alcohol deproteinization; and (iii) completely digested within 3 hours by incubation with 66 μg of type II snake-venom phosphodiesterase and 66 μg of bovine pancreatic deoxyribonuclease per milliliter.

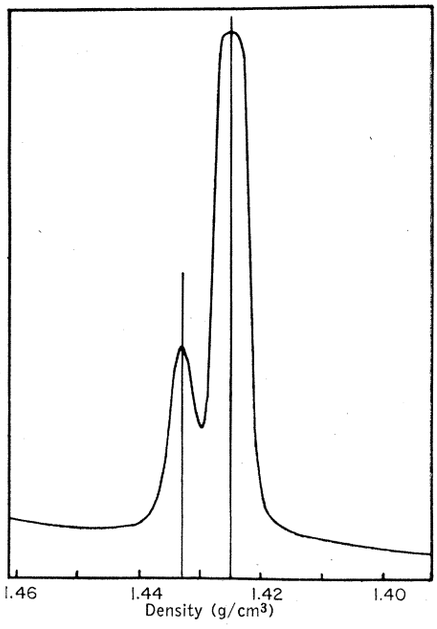


Fig. 3. Buoyant densities in Cs_2SO_4 of DNA extracted from *Bacillus cereus* spores 1 minute after initiation of germination. Densities were measured with a pycnometer.

The satellite could be partially denatured DNA. However, it was never detected in DNA preparations isolated by similar methods from cells in the mid-log phase of growth. To further test this possibility, DNA preparations with and without satellite DNA were centrifuged in alkaline CsCl (*12*). The shifts in buoyant density of both the major and minor components after alkaline denaturation were typical of double-stranded DNA (Fig. 2). Similar results were obtained with preparations denatured by heating and quick cooling. Thus, under strong denaturing conditions, two components were found only in those preparations which had originally contained two components, an indication that the satellite DNA band was not partially denatured DNA of the major component.

The buoyant densities of 1.425 g cm^{-3} for the main component and 1.433 g cm^{-3} for the satellite DNA in Cs_2SO_4 were determined by pycnometric measurements (Fig. 3). The close correlation of these figures with those predicted from their respective guanosine plus cytosine contents (*10*) suggests that neither the main DNA component nor the satellite DNA was either glucosylated or denatured.

The percentage of the total DNA in the satellite peak was measured as a function of time after the beginning of germination with the aid of the integrating Joyce Chromoscan densitometer. In this system, cells had begun to form the first cross walls 60 minutes after germination was begun and were clearly at the two-cell stage at 75 minutes. The satellite DNA was thus present in maximum amount shortly after initiation of germination and gradually disappeared during cell division. The cause of its disappearance is unknown. The fact that no detectable shift occurred in the buoyant density of the major component as a result of the disappearance of the satellite DNA suggests that it was not incorporated *in toto* into the major component.

The satellite was completely absent from vegetative cells, and could not be detected in the sporangium of cells at late sporulation. Since the satellite has thus far been found only in dormant spores and spores undergoing outgrowth, its function is possibly related to spores. Although attempts with mitomycin C and hydrogen peroxide have not demonstrated the induction of a temporate phage containing nucleic acid with the properties of this satellite, this possibility cannot be eliminated. Neither can the possibility that local polyteny is the source of the satellite DNA be ruled out. The presence of a second DNA component in spores and outgrowing cells of *B. cereus* T, however, is consistent with the hypothesis (*13*) that sporulation is controlled by episomal elements present in variable amounts during the life cycle of *Bacillus* species (*14*).

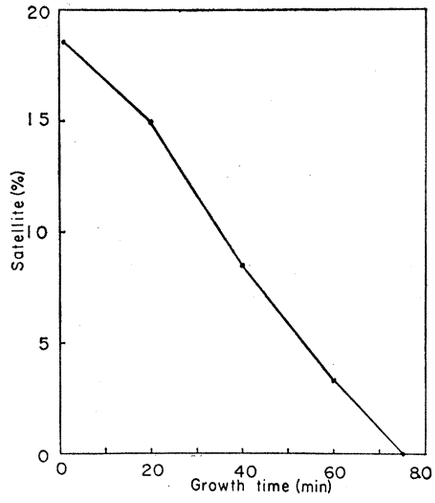


Fig. 4. Percentage of total DNA in the satellite as a function of time, measured with the integrating Joyce Chromoscan densitometer.

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