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3. W. Gertsch examined the spider and determined that the species is new; a description of it is being prepared, and specimens will be deposited in the Museum of Comparative Zoology, Cambridge, Mass. 02138.
4. The study was done from January to March, and during July 1977 on the Melendez campus of the Universidad del Valle, Cali, Colombia, and included more than 25 hours of direct observation at night. The campus is in the midst of extensive sugar cane fields. The spiders, all mature females, were in a 30-m stretch of a barbed wire fence (3 m high) in the middle of a large (about 100 by 150 m) field of grass and weeds. Their behavior and that of their prey was observed both by the light of several street lights about 70 m away, and with a headlamp. The headlamp had no appreciable effect on the behavior of the prey, and typically was lit only after prey had approached the spider. Winds during observation periods were light (0 to 6 km/hour); they were most frequently from the south, but changed erratically to other points of the compass.
5. The ball was collected with a minimum of disturbance by cutting it loose as it swung free the moment the spider finished producing it. In such cases the spider usually walked back and forth several times on the horizontal line before settling down in its attack posture.
6. A ball placed on a piece of filter paper which was then placed in a screen cage (0.7 by 0.7 by 0.7 m) containing six male *S. frugiperda* moths (captured less than 2 hours before as they hovered near a spider) also failed to elicit any response. Balls are eaten about every 30 minutes and then replaced, the probable reason for this being that the ball loses its stickiness as it dries out.
7. The exception was a spider from which I had taken a ball; after assuming a predatory stance for about 15 minutes, she resumed the resting position, but a moth hovered nearby off and on for the next 3 minutes.
8. Horizontal sticky traps were placed about 50 cm below the spiders to collect discarded prey, and checked daily. Prey items were not greatly damaged, but were easily distinguished from other insects by their silk wrapping.
9. Dr. E. L. Todd kindly identified specimens of the moths. It was not possible to determine the *Leucania* to species, and more than one species may have been present. Specimens have been deposited in the U.S. National Museum, Washington, D.C. 20560, and the Departamento de Biología, Universidad del Valle, Cali, Colombia.
10. The traps consisted of arrays of sticky nylon monofilament supported by aluminum frames (23 by 32 cm). (W. G. Eberhard, *Bull. Br. Arach. Soc.*, in press). They were hung in the fence at the same height as the spiders.
11. Moths were collected in the adjacent field both by spotting resting individuals with a headlamp (about 10 hours on four nights) and at a light trap (6 hours on two nights). The numbers given are certainly underestimates of the numbers of species actually present. Specimens are deposited in the Departamento de Biología, Universidad del Valle, Cali, Colombia.
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15. I noted 86 moth approaches in 517 minutes on 12 different nights. A moth was scored as having approached if it hovered for more than 3 seconds within 1 m downwind of a spider.
16. Of 82 approaches, 20 resulted in strikes by the spider, and 40 percent of these strikes were successful.
17. After 54 spider nights, 121 discarded moths were found in the traps under the spiders. These counts are conservative since winds and spider movements probably caused some discarded prey to fall free of the traps. Spiders took about 60 to 90 minutes to consume each moth caught, and did not hunt as they fed.
18. Moths averaged 60 mg (wet weight) ($N = 9$). The spiders' weights fluctuated as a result of periodic production of egg masses weighing about 350 mg; they probably averaged about 750 mg.
19. M. Robinson and B. Robinson, *Zool. J. Linn. Soc.* **49** (No. 4), 345 (1970); mature female *A. argentata* weigh about 500 mg.
20. Supported by the Comité de Investigaciones, Universidad del Valle. I thank M. Birch, H. Dahners, and A. Shapiro for help with the moths, and M. J. W. Eberhard, Y. Lubin, and R. Silberglied for criticizing the manuscript.

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Characterization of Bacterial Growth by Means of Flow Microfluorometry

Abstract. *By means of flow microfluorometry, the protein and nucleic acid contents of individual bacterial cells may be measured at the rate of several thousand cells per second. Accumulation of such information over a few minutes yields the composition distribution of the microbial population. These distributions have been determined at different times during batch growth of* Bacillus subtilis, *and the results indicate that the variance of cell composition decreases as the population passes through the exponential into the stationary phase. The relative abundance of endospores and vegetative cells as well as the protein distributions of these subpopulations may be readily determined from flow microfluorometry data. Experimental access to such details of microbial population dynamics should foster improved understanding of cell growth, spore germination, and spore formation kinetics.*

Experimental methods for observing and characterizing a population of growing microorganisms may be divided into two broad categories: those which determine properties averaged over a large number of cells and those which measure individual cell characteristics. Methods such as turbidimetry and conventional biochemical analyses belong to the first class, and have the advantage of providing data on relatively large-sized samples. However, these techniques by definition provide no information on differences in size and composition among individual cells in the population.

Unless special methods are used to obtain synchrony, a pure bacterial culture is a heterogeneous population in which young cells and old cells, single cells and cell chains, and vegetative cells and endospores influence each other through their interaction with a common environment. To understand these interactions one needs information on the distributions of various classes of the cell population under different growth conditions. Statistically significant measurements of these distributions for classification according to size have been obtained according to the Coulter principle for a number of bacteria, including *Escherichia coli*, *Azotobacter agilis*, and *A. vinelandii* (*1*).

The laser flow microfluorometer (FMF) (*2*), which has been applied almost exclusively to mammalian cells in the past (*3*), provides a convenient means of rapidly measuring the protein and nucelic acid content of individual cells. In this instrument cells stained with specific fluorescent dyes flow at rates of 500 to 3000 cells per second through a 0.5-watt continuous argon laser (488 nm). The resulting fluorescence, which is proportional to cellular content of the stained component, is detected by photomultiplier tubes for storage in a computer or multichannel pulse height analyzer.

We have used the FMF to study changes, during batch growth, in the composition distributions of *Bacillus subtilis* ATCC 6051a. The organism was grown at 34°C in a semisynthetic medium described by Jensen (*4*), modified by using 2 percent (weight to volume) glucose instead of maltose. Previously adapted midstationary cells were inoculated into 100 ml of medium contained in 250-ml Erlenmeyer flasks and placed on a rotary action shaker at 300 rev/min. Growth was monitored by means of a Klett-Summerson colorimeter (blue filter) (see Fig. 1, inset). The lag phase observed under our culture conditions lasted about 3 hours, and subsequent exponential growth ceased after approximately 4 hours. At intervals over a 9-hour period, samples of the cell population were harvested, fixed in 70 percent ethanol, and stained with either fluorescein isothiocyanate (FITC, specific for protein) or propidium iodide (PI, specific for double-stranded nucleic acid) (*5*).

Figure 1 illustrates the changes in the population's nucleic acid distribution which occur during batch growth. The average cellular nucleic acid content, which is proportional to the first moment of these distributions, decreases as the bacterial population moves from the early exponential phase (Fig. 1A) to the exponential-stationary transition (Fig. 1B) and finally to the stationary (Fig. 1C) phase of growth. These trends are con-

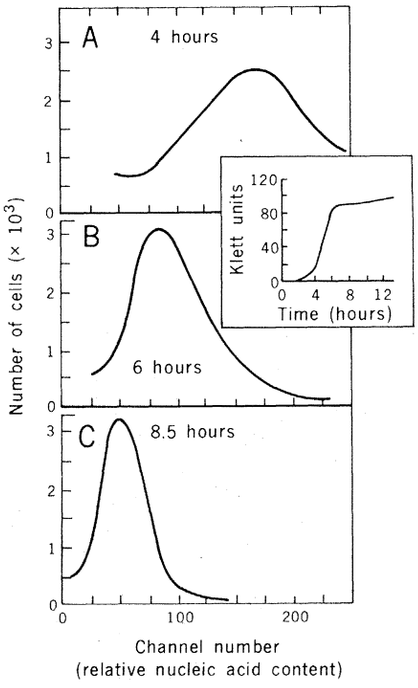

Fig. 1. Changes in the nucleic acid distribution with time in batch growth of *B. subtilis*. Elapsed time since inoculation is shown with each set of data. Data are obtained as numbers of cells (ordinate) corresponding to relative double-stranded nucleic acid content (channel numbers; abscissa). Counts in the first few channels were deleted from this figure because they resulted from electronic or background fluorescence noise. Separation of the bacterial fluorescence data shown here from instrument noise requires optimum laser alignment and tuning and, occasionally, imposition of a coincidence gate for acceptance of photomultiplier tube signals.

sistent with those reported previously for average nucleic acid content determined by conventional analyses (*6*). However, the FMF data provide additional information. For example, Fig. 1 shows that the variance of cell nucleic acid content also decreases as the microbial population enters the stationary phase.

The potential advantages of composition distribution information as opposed to average composition data are revealed in the sequence of population protein distributions shown in Fig. 2. The inoculum, which consists mostly of free spores, has a very narrow protein distribution with a relatively small mean value (Fig. 2A). After 4 hours of growth, the midexponential bacterial population consists entirely of vegetative cells with much larger mean protein content and protein variance (Fig. 2B). The variance and mean protein content have both decreased by the end of the exponential phase (Fig. 2C), and we obtain a bimodal protein distribution during the stationary phase (Fig. 1D).

Comparison of Fig. 2, A, C, and D, reveals that the location of the first maximum in the protein distribution at 8.5 hours corresponds extremely closely to the single peak for spores in the inoculum, while the second maximum in Fig. 2D appears at a slightly smaller protein content than the maximum of Fig. 2C. Thus we conclude that the population at 8.5 hours consists of both free spores (the first peak) and vegetative cells (the second peak) with less disperse and smaller average protein contents than exponential phase cells. This result has been verified qualitatively by microscopic examination. The observed 8.5-hour nucleic acid distribution is not bimodal, because it is not possible to distinguish spore nucleic acid fluorescence from background optical and electronic noise in these FMF experiments.

The FMF data such as those in Fig. 2D include the relative numbers of vegetative cells and free endospores as well as the statistical properties of both subpopulations. We have decomposed the bimodal protein distribution data for 8.5 hours by least-squares optimization of the parameters in a linear combination of two gamma distributions, and the resulting fit is shown as the dashed curve in Fig. 2D. Examination of the two underlying distributions and their relative weights reveals that free endospores constitute 13.5 percent of the population, that the ratio of mean spore protein content to mean vegetative cell protein content is 0.37, and that the standard deviation for vegetative cell protein content is 0.85 that of the free spores.

Such information on subpopulation characteristics is not accessible from conventional protein determinations. Data on average population composition also provide no information on the formation and disappearance of the cell chains that have been observed in experiments in which different culture conditions have been used. The presence of cell chains is manifested in FMF data by changes in shape in the tail of the distribution; in some cases these changes are sufficiently pronounced to introduce additional modes. In both situations the decomposition calculations discussed above can be used to determine the relative numbers and composition characteristics of cell chains. While these processes can be monitored by means of microscopy, accurate quantification is difficult and subject to considerable statistical uncertainty because of the small sample size. Further FMF studies focusing on microbial population changes on a finer time scale than in the present experiment should provide new insights into the kinetics and interactions involved in bacterial spore germination and vegetative cell growth, reproduction, and sporulation. Also, the technique may be readily extended to studies of unicellular algae, yeast, and protozoan populations.

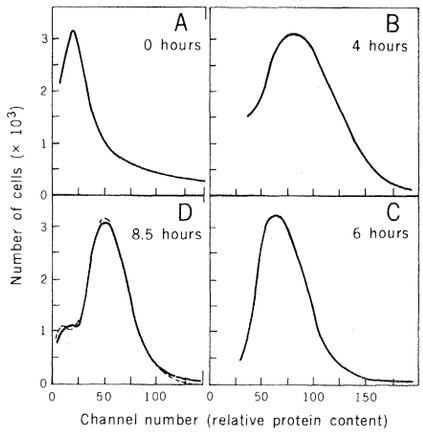

Fig. 2. Evolution of the protein (FITC fluorescence) distribution of the *B. subtilis* population during batch growth. Data display and truncation are as in Fig. 1. The dashed line in (D) is the result of least-squares fitting of two superimposed gamma distributions to the experimental data. The fitted curve does not show over intervals where it is indistinguishable from the data on the scale used here.

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