JOURNAL OF BACTERIOLOGY, Oct. 1976, p. 506-509

Vol. 128, No. 1
Printed in U.S.A.

Light-Induced Inhibition of Sporulation in *Bacillus licheniformis*

C. PROPST-RICCIUTI* AND L. B. LUBIN

GTE Laboratories, Inc., Biological Sciences, Waltham, Massachusetts 02154

Received for publication 13 July 1976

Sporulation of *Bacillus licheniformis* is inhibited by broad-spectrum light. This phenomenon is intensity dependent and is a near-ultraviolet and blue light effect.

Light has a wide variety of effects on microorganisms (3, 14, 20). The majority of studies with bacteria, however, have centered around the growth-delaying, mutagenic, and lethal effects of ultraviolet radiation (10, 20). Except short-wavelength visible radiation, which has been studied because of its photoreactivating (5, 20) and mutagenic (2, 5, 9) potential, very few investigations have been made to determine the influence of visible and near-visible light on procaryotes. Recently, it has been shown that light with wavelengths longer than 400 nm affects certain metabolic and physiological processes in bacteria. Tumbling movement and, subsequently, cell paralysis occur when bacteria are exposed to visible light (18). In addition, respiration, permeation, and adenosine 5'-triphosphate (ATP) production can be influenced by light (1, 4, 13).

While the effects of light on bacteria were being studied, it was observed that the absolute number of spores produced by *Bacillus licheniformis* ATCC 10716 continually exposed to broad-spectrum irradiation was less than the number of spores produced by comparable dark-grown cultures. In these experiments bacteria were grown overnight in nutrient broth at 37°C under previously described conditions (15) and spores (i.e., heat-resistant colony-forming units) were determined by the method of Taber and Freese (17). Broad-spectrum lighting was provided by one of three types of fluorescent lamps, whose electromagnetic spectra have been described previously (see reference 16 for a description of types A, B, and D). Sporulation inhibition varied depending on the conditions of lighting and other environmental parameters. For one continuous broad-spectrum light source (A) at an intensity of 1,980 $\mu W/cm^2$, the light/dark ratio of spores (per ml) averaged 0.42 ± 0.15. Comparable results were obtained with the other light sources (B, D). In additional experiments, broad-spectrum light (A, 1,800 $\mu W/cm^2$) was shown to inhibit sporulation in *Bacillus megaterium* ATCC 19213, *Bacillus subtilis* ATCC 6051, and *Bacillus cereus* CB, with light/dark spore ratios, respectively, of 0.26 ± 0.19, 0.13 ± 0.12, and 0.58 ± 0.35. In each case data are averages of a minimum of three experiments, ±1 standard deviation.

One possible explanation for dark-grown cultures producing more spores than cultures continually exposed to light is that dark-grown cultures initially produce more cells than light-grown cultures. This explanation was eliminated by coordinately comparing the growth and sporulation of *B. licheniformis* under continuous broad-spectrum irradiation. Figure 1 shows that dark-grown bacteria do show slightly more growth than light-grown cultures, as expected from previous studies (10, 11). The average (±1 standard deviation) light/dark growth ratio at stationary phase was 0.77 ± 0.05 for three experiments. When, however, the number of spores produced by the stationary-phase cultures in Fig. 1 at 7 h was determined as a function of the number of viable cells present, the light-grown culture showed 0.074% sporulation (3.8×10^4 spores/5.3×10^7 cells), whereas the dark-grown cultures showed 0.19% sporulation (1.2×10^5 spores/6.5×10^7 cells). The light/dark spore ratios, corrected for the number of available cells measured by viable count and by optical density, respectively, were 0.39 and 0.40. Thus, the difference in cell numbers in light- and dark-grown cultures does not account for the difference in spores produced by these cultures. Control experiments have also shown that both the growth and sporulation responses are not due to irreversible effects of light on the growth medium itself. Dark-grown cells in media pretreated with light before inoculation show the same growth pattern and amount of sporulation as dark-grown cells whose media were not preexposed to light.

The relationship between sporulation and broad-spectrum light was further investigated

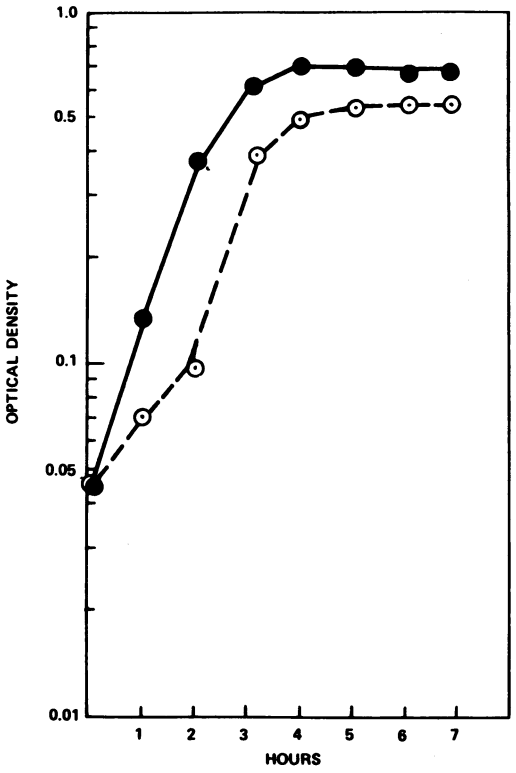

FIG. 1. *Bacterial growth under light and dark conditions. Bacteria were dispersed in broth and subdivided into two cultures for growth under continuous light (broad spectrum A, 1,850 μW/cm²) or dark conditions. Growth was measured as optical density at 540 nm. Symbols:* (●) *dark growth;* (○) *light growth.*

using variable intensities of irradiation (Fig. 2). The relationship between these parameters is best defined by the equation $S = e^{-m\phi}$, where S is the fraction of sporulation, e is the base of the natural logarithms, m is the slope of the line, and ϕ is the light intensity. For the experimental conditions defined here, m has a value (plus or minus standard error) of 5.52 (±0.45) $\times 10^{-4}$ cm² μW^{-1}.

To determine which specific regions of the light spectrum were responsible for the inhibition of sporulation, cultures were exposed to various narrow bandwidth fluorescent lights having maxima at 371, 419.5, 550, 660, and 750 nm (15, 16), and the level of sporulation under each light at 440 μW/cm² was measured. Preliminary experiments had shown that a semilogarithmic plot of spore fraction versus intensity of light was linear from 0 to 440 μW/cm² for the 371-nm light and from 0 to 780 μW/cm² for the 419.5-, 550-, 660-, and 750-nm lights, where 440 and 780 μW/cm² were the maximum intensities tested for the 371-nm and other lights, respectively. The results of correlating sporulation inhibition with light wavelength are shown in Fig. 3 and indicate that light inhibition of sporulation is primarily associated with shorter wavelengths of the near-ultraviolet and blue regions of the spectrum. Longer wavelengths either have no effect on sporulation or may enhance sporulation slightly.

The inhibition of sporulation observed under shorter wavelengths of light is not due to a permanent block in sporulation. As shown in Fig. 4, cells continuously exposed to 371-nm light will eventually sporulate to the same extent as dark-grown cells; however, complete sporulation of light-grown cultures takes a longer period of time.

At the present time, neither the primary physiological processes nor the photosensitizing molecules involved in light delay of sporulation have been identified. It is known that exposure

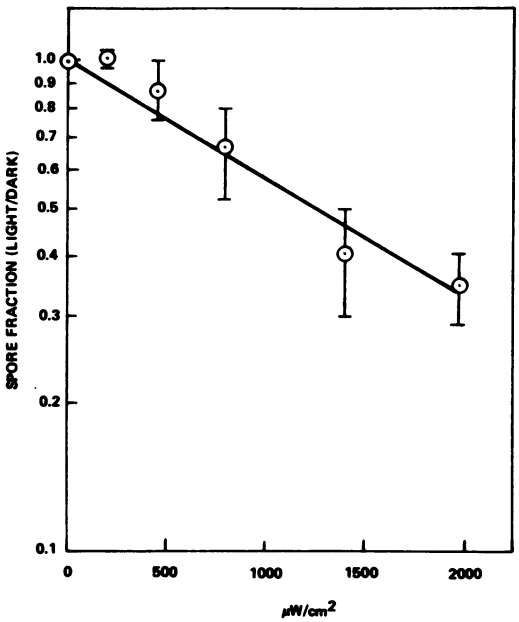

FIG. 2. *Effect of light intensity on sporulation inhibition. Bacteria were dispersed in broth and subdivided into samples for growth under continuous light (broad spectrum A) or dark conditions for 18 h. Duplicate or triplicate samples were run for each intensity in each experiment. Replicate samples within each experiment were pooled before titration of spores. Data are presented as the spore fraction, i.e., the number of spores produced by light-grown cultures corrected for growth (optical density at 540 nm), divided by the number of spores produced by dark-grown cultures corrected for growth. Data are averages from different experiments (generally four): vertical lines indicate 1 standard deviation.*

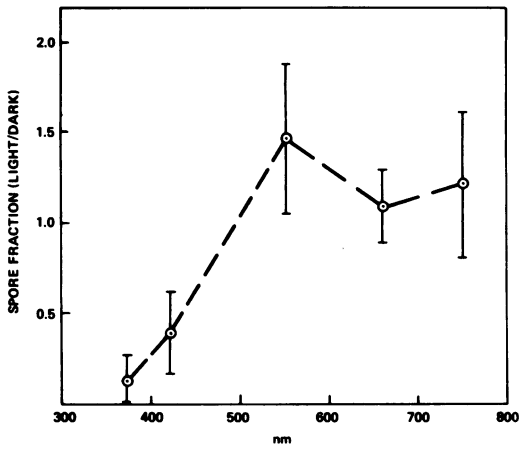

FIG. 3. *Effect of light wavelength on sporulation inhibition. Bacteria were dispersed in broth and subdivided into samples for growth under continuous light at the indicated wavelengths at 440 μW/cm² and in the dark. Duplicate samples were run for each condition. Incubation was for 18 h, and replicate samples within each experiment were pooled before titration of spores. Spore fraction is defined as in Fig. 2. Data are an average of three experiments; vertical lines indicate 1 standard deviation.*

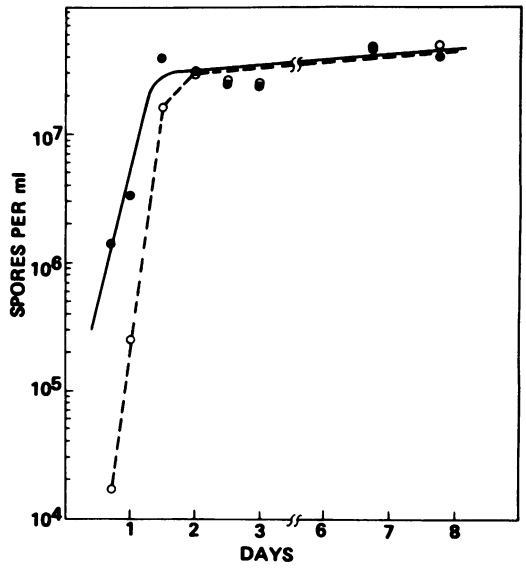

FIG. 4. *Production of spores by cultures exposed to 371-nm light. Bacteria were dispersed in broth and subdivided into cultures for growth under continuous light (371 nm, 500 μW/cm²) or dark conditions. Data are expressed as spores per milliliter, since growth inhibition (see Fig. 1) was not observed under 371-nm light. Symbols: (●) spores produced in dark; (○) spores produced in light.*

of bacteria to light similar to that used in these studies decreases respiration and ATP levels (4, 10, 13). ATP is required both as an energy source and as a precursor of regulatory nucleotides for sporulation. Mutants with defective enzymes in their citric acid cycle, as well as cytochrome mutants, sporulate poorly as a result of their inability to produce enough ATP (7, 12, 17). The effect of light on sporulation might therefore be directly due to curtailed respiration and/or ATP production. Since the activity of certain enzymes believed to be important in coupling oxidative energy to transport is decreased by light and permeation of specific amino acids is inhibited (4, 12), an alternative explanation would be that light acts on the permeation system in such a way as to lower the concentration of specific amino acids or other metabolites required for sporulation in either a structural or regulatory capacity. Cystine (cysteine), a major constituent of the spore coat, is present in spores in amounts that are three to five times higher than those found in vegetative cells (8).

The fact that delay of sporulation occurs only at shorter wavelengths of light indicates that the molecule(s) responsible for this phenomenon must absorb light in this region of the spectrum. Of the numerous molecules that meet this criterion, special consideration must be given to the quinones, flavoproteins, and cytochromes as potential photosensitizers in sporulation inhibition. Not only do these molecules absorb light in the appropriate regions of the spectrum, but also, they are known to be destroyed both in vitro and in vivo by near-ultraviolet and blue light (4, 6, 10, 11, 19), and they make up essential parts of the respiratory complex of the vegetative cell as well.

We thank Peter Cukor, Joel Krugler, and Michael Seibert for many helpful discussions. In addition, we thank Joel Krugler for statistical analysis of our data.

LITERATURE CITED

1. **Barran, L. R., J. Y. D'Aoust, J. L. Labelle, W. G. Martin, and H. Schneider.** 1974. Differential effects of visible light on active transport in *E. coli*. Biochem. Biophys. Res. Commun. 56:522–528.
2. **Cabrera-Juarez, E., and M. Espinosa-Lara.** 1974. Lethal and mutagenic action of black light (325 to 400 nm) on *Haemophilus influenzae* in the presence of air. J. Bacteriol. 117:960–964.
3. **Carlile, M. J.** 1965. The photobiology of fungi. Annu. Rev. Plant Physiol. 16:175–202.
4. **D'Aoust, J. Y., J. Giroux, L. R. Barran, H. Schneider, and W. G. Martin.** 1974. Some effects of visible light on *Escherichia coli*. J. Bacteriol. 120:799–804.
5. **Eisenstark, A.** 1970. Sensitivity of *S. typhimurium* recombinationless (rec) mutants to visible and near-visible light. Mutat. Res. 10:1–6.
6. **Epel, B. L.** 1973. Inhibition of growth and respiration by visible and near-visible light. Photophysiology 8:209–229.

7. **Freese, E., P. Fortnagel, R. Schmitt, W. Klofat, E. Chappelle, and G. Picciolo.** 1969. Biochemical genetics of initial sporulation stages, p. 82–101. *In* L. L. Campbell (ed.), Spores IV. American Society for Microbiology, Bethesda, Md.
8. **Hanson, R. S., J. A. Peterson, and A. A. Yousten.** 1970. Unique biochemical events in bacterial sporulation. Annu. Rev. Microbiol. 24:53–90.
9. **Hollaender, A.** 1943. Effect of long ultraviolet and short visible radiation (3500 to 4900Å) on Escherichia coli. J. Bacteriol. 46:531–541.
10. **Jagger, J.** 1972. Growth delay and photoprotection induced by near-ultraviolet light, p. 383–401. *In* U. Gallo and L. Samtamiria (ed.), Research progress in organic-biological and medical chemistry, vol. III. North-Holland Publications, Amsterdam.
11. **Kashket, E. R., and A. F. Brodie.** 1962. Effects of near-ultraviolet irradiation on growth and oxidative metabolism of bacteria. J. Bacteriol. 83:1094–1100.
12. **Klofat, W. G., G. Picciolo, E. W. Chappelle, and E. Freese.** 1969. Production of ATP in normal cells and sporulation mutants of *B. subtilis*. J. Biol. Chem. 244:3270–3276.
13. **Lakchaura, B., T. Fossum, and J. Jagger.** 1976. Inactivation of adenosine 5'-triphosphate synthesis and reduced form nicotinamide adenine dinucleotide dehydrogenase activity in *Escherichia coli* by near-ultraviolet and violet irradiations. J. Bacteriol. 125:111–118.
14. **Leach, C. M.** 1971. A practical guide to the effects of visible and ultraviolet light on fungi. Methods Microbiol. 4:609–664.
15. **Propst-Ricciuti, C., and C. Kenny.** 1976. The effect of light on the growth of β-lactam antibiotic producing fungi, p. 233–240. *In* L. A. Underkofler (ed.), Developments in industrial microbiology, vol. 17. Impressions, Ltd., Gaithersburg, Md.
16. **Seibert, M., P. J. Wetherbee, and D. D. Job.** 1975. The effects of light intensity and spectral quality on growth and shoot initiation in tobacco callus. Plant Physiol. 56:130–139.
17. **Taber, H., and E. Freese.** 1974. Sporulation properties of cytochrome *a*-deficient mutants of *Bacillus subtilis*. J. Bacteriol. 120:1004–1011.
18. **Taylor, B. L., and D. E. Koshland, Jr.** 1975. Intrinsic and extrinsic light responses of *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. 123:557–569.
19. **Werbin, H., B. Lakchaura, and J. Jagger.** 1974. Near-ultraviolet modification of *E. coli* ubiquinone *in vivo* and *in vitro*. Photochem. Photobiol. 19:321–328.
20. **Zelle, M. R., and A. Hollaender.** 1955. Effects of radiation on bacteria, p. 365–430. *In* Radiation biology. McGraw-Hill Book Co., New York.