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Light Sensitivity of Methanogenic Archaebacteria

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Representatives of four families of methanogenic archaebacteria (archaea), *Methanobacterium thermoautotrophicum* ΔH, *Methanobacterium thermoautotrophicum* Marburg, *Methanosarcina acetivorans*, *Methanococcus voltae*, and *Methanomicrobium mobile*, were found to be light sensitive. The facultative anaerobic eubacteria *Escherichia coli* and *Salmonella typhimurium*, however, were tolerant of light when grown anaerobically under identical light conditions. Interference filters were used to show that growth of the methanogens is inhibited by light in the blue end of the visible spectrum (370 to 430 nm).

Methanogenic archaebacteria (archaea) are oxygen-sensitive organisms which convert H_2 and CO_2 to methane (3, 6, 12). It has been shown previously that methanogenic bacteria are sensitive to mutagenic effects of UV irradiation (similar to the UV sensitivity of *Escherichia coli*) (7). This lethal effect was reversed by relatively short (2 to 3 h) exposure to white light. The purified DNA photolyase was found to contain an intrinsic 8-hydroxy-5-deazaflavin (coenzyme F420) as a chromophore (8). In addition to O_2 and UV light, methanogens are known to be sensitive to 2-bromoethanesulfonic acid (15), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (9), chloral hydrate [$CCl_3CH(OH)_2$] (17), and the methane analogs CCl_4, $CHCl_3$, and CH_2Cl_2 (2, 14). In this communication, we describe the sensitivity of methanogens to continuous exposure to light in the visible region of the spectrum.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Methanobacterium thermoautotrophicum* ΔH (DSM 1053) and Marburg (DSM 2133) were grown in general salts medium no. 3 as previously described (1), with the following modifications: no yeast extract was added, and 9 g of NaCl and 5 mg of $NiCl_2$ were added per liter. *Methanosarcina acetivorans* (DSM 2834) was grown in general salts medium with 0.3% methanol, which it utilizes as a carbon and energy source. *Methanomicrobium mobile* (DSM 1539) was grown in a partially defined medium which contained Casamino Acids and boiled cell extract from *M. thermoautotrophicum* ΔH (16). *Methanococcus voltae* (DSM 1537) was grown as described previously (19). *E. coli* (strain DMS 7118) and *Salmonella typhimurium* (strain LT 2) were grown in a 1:1 mixture of general salts medium and nutrient broth (1 g of maltose, 2.5 g of yeast extract, 10 g of tryptone, 5 g of NaCl, and 10 ml of 1 M $MgSO_4 \cdot 7H_2O$ per liter). All organisms were anaerobically inoculated into tubes containing 5 ml of medium as described previously (1), each inoculum being 1 ml from a log-phase culture. Growth studies were performed in triplicate, and the absorbance readings were averaged. Growth was monitored by measuring turbidity at 660 nm with a Bausch and Lomb Spectronic 20 spectrophotometer; an 18-mm light path was used. Anaerobic roll tubes were prepared for enumeration of *M. voltae*, the medium being general salts medium supplemented with 0.2% yeast extract and 2% (wt/vol) Noble agar (Difco). Filter-sterilized titanium citrate [Ti(III) citrate; final concentration, 1 mM] was added to the agar medium as an additional reductant after the medium was sterilized. The atmosphere in each tube was pressurized with H_2 and CO_2 (80:20) to 200 kPa, and tubes were incubated at 30°C.

To ensure a constant temperature during illumination, each culture tube was positioned horizontally in a water bath maintained at the optimal growth temperature (60°C for *M. thermoautotrophicum*, 30°C for *M. voltae*, and 37°C for *M. acetivorans*, *M. mobile*, *E. coli*, and *S. typhimurium*). The temperature of the contents of an illuminated tube did not change more than 0.5°C. Illumination of a culture had no effect on the pH of the medium. In addition, illumination of sterile medium for 3 days had no effect on the subsequent ability of *M. acetivorans* or *M. thermoautotrophicum* to grow in the dark on this medium. All cultures were gently shaken except for *M. acetivorans* and *M. mobile*, which grow well without agitation.

Light source and interference filters. Incandescent tungsten "soft white" bulbs of different wattages (60 W, 100 W, and 150 W) were used. A photometer (Li-Cor model LI-185B with an LI-2005B pyranometer sensor) was used to measure the fluence rates (in watts per square meter). Where indicated, a 40-W General Electric blue fluorescent bulb (F40B) was used in place of the tungsten bulbs. The photometer measures photons in the 400- to 700-nm range. Therefore, the fluence rate of the blue light is smaller than that of the incandescent bulbs, which emit photons over a larger range. Tubes in the light were continuously irradiated, unless indicated otherwise. Interference filters were purchased from Oriel Corporation (Stratford, Conn.). The width of the transmission curves at 50% of the maximum transmission was 55 nm for the 398-nm filter (P/N 57521) and 71 nm for the 500-nm filter (P/N 57550).

Microscopy. An Olympus system microscope (model BHS; Olympus, Overland Park, Kans.) was employed for all microscopic observations. The reflected-light fluorescence attachment (model BH2-RFL) was also from Olympus.

Coenzyme F420. Coenzyme F420 [ε_{420} = 40,000 M^{-1} cm^{-1}; a 7,8-di-demethyl-8-hydroxy-5-deazariboflavin 5′-phosphate, which has *N*-(*N*-L-lactyl-γ-L-glutamyl)-L-glutamic acid attached to it via a phophodiester linkage] was purified to homogeneity from cells of *M. thermoautotrophicum* ΔH by the method of Eirich et al. (4).

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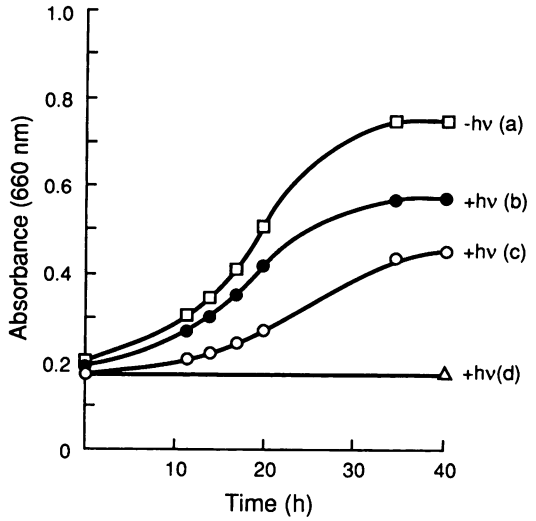

FIG. 1. *M. acetivorans* grown in the presence of various light intensities. The growth curves are labeled as follows: a, culture incubated in the dark (wrapped in aluminum foil); b, culture placed 14 cm from a 60-W incandescent bulb (1.1×10^2 W m^{-2}); c, culture placed 7 cm from a 60-W incandescent bulb (4.5×10^2 W m^{-2}); and d, culture placed 8 cm from a 100-W incandescent bulb (7.2×10^2 W m^{-2}). +hν, incubated in light; −hν, incubated in darkness.

RESULTS

A 100-W bulb placed 8 cm from the samples (equivalent to 7.2×10^2 W m^{-2}) completely inhibited growth of *M. acetivorans* and *M. thermoautotrophicum* ΔH. *M. acetivorans* exposed to light initially showed a minimal increase in absorbance ($A_{660} = 0.1$) but did not show an increase after 24 h. *M. thermoautotrophicum* ΔH, however, decreased in turbidity because of cell lysis. A 150-W bulb placed 8 cm from the tubes (equivalent to 8.7×10^2 W m^{-2}) was required to stop all growth of *M. thermoautotrophicum* Marburg. In the medium employed, the anaerobic growth of *E. coli* and *S. typhimurium* under relatively intense illumination (7.2×10^2 W m^{-2}) was the same as the growth of cells in the dark, and the final absorbance of illuminated and control tubes was the same for each eubacterium (data not shown).

M. mobile, one of the more fastidious methanogens, was also found to be light sensitive. *M. mobile* reached a final absorbance of 0.21 in the dark and 0.0 in the presence of light (General Electric blue fluorescent, 8 W m^{-2}). The effect of light intensity on growth was demonstrated for *M. acetivorans* by measuring growth at differing light intensities with incandescent bulbs. As seen in Fig. 1, cells kept in the dark had a doubling time of 11.7 h. A slower growth rate (doubling time = 13.6 h) was seen at 1.1×10^2 W m^{-2} (60-W bulb at 14 cm). An even greater inhibition (doubling time = 17.8 h) was seen at 4.5×10^2 W m^{-2} (60-W bulb at 7 cm). As previously mentioned, an intensity of 7.2×10^2 W m^{-2} completely inhibited growth of this methanogen.

Methanogens can be identified on the basis of their autofluorescence (due to intrinsic coenzyme F420) under UV illumination (10). A culture of *M. acetivorans* was illuminated for 3 days under such intense illumination as to completely inhibit growth. There was virtually no difference in the intensity or length of autofluorescence of the illuminated cells compared with that of the cells grown in the dark, suggesting that coenzyme F420 (or a derivative of it) may not be involved in the sensitivity of methanogens to light.

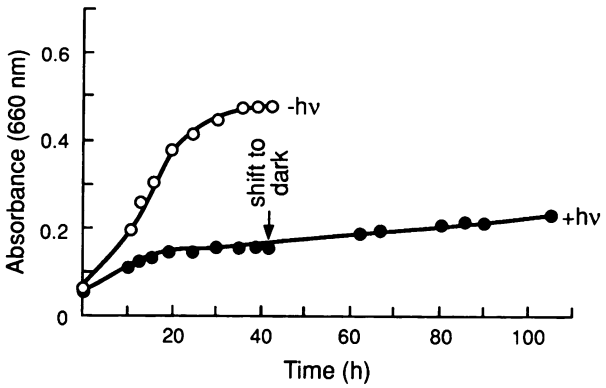

FIG. 2. *M. acetivorans* grown in the presence and absence of light. A 100-W incandescent bulb was used to illuminate the tubes (7.2×10^2 W m^{-2}). At the time indicated by the arrow, the illuminated tubes were wrapped in aluminum foil. +hν and −hν, same as for Fig. 1.

Illumination of cultures decreased the number of viable cells. To assess the possibility that the cells were killed upon illumination, two cultures of *M. acetivorans* were grown for 40 h, one in the presence and one in the absence of light. At this time, the illuminated tube was shifted to the dark and cell growth was monitored by measuring absorbance versus time. As shown in Fig. 2, the absorbance increased only slightly, indicating that light-exposed cells were poorly viable or nonviable.

An experiment was designed to enumerate the number of viable cells of *M. voltae* subsequent to light treatment. Three tubes of liquid medium were prepared. One tube contained only an inoculum which was immediately diluted into roll tubes without further treatment. Cells in a second tube were kept in the dark and grew normally. The third tube was inoculated and irradiated with a blue fluorescent light (8 W m^{-2}). After 26 h of incubation at 30°C, samples of the cultures from the second and third tubes were diluted and plated into roll tubes. The tubes were incubated in the dark, and colonies were counted after 5 days. The results are as follows. The tube containing only the inoculum was found to have 6.87×10^5 cells per ml ($A_{660} = 0.025$; standard deviation = 2.0×10^5). The tube incubated in the dark contained 2.25×10^6 cells per ml (final $A_{660} = 0.13$; standard deviation = 7.9×10^5). The tube that was illuminated contained 9.59×10^4 cells per ml (final $A_{660} = 0.02$; standard deviation = 2.4×10^4). We therefore observed a loss of cell viability of the culture in the irradiated tube compared with that of the original inoculum (approximately 86%).

Blue light inhibits methanogens. Interference filters were used to show that methanogens are inhibited by light in the blue region of the visible spectrum. As Fig. 3 indicates, light that had passed through the 398-nm filter completely inhibited growth, whereas light that had passed through the 500-nm filter had only a moderate inhibitory effect on the growth of *M. acetivorans*.

Red fluorescence. Epifluorescence microscopy of *M. acetivorans* grown in the presence of light that had passed through the 500-nm interference filter revealed that up to 20% of the cells fluoresced brilliant red rather than blue green. Upon further incubation of the culture in the dark, red fluorescent cells were not observed, suggesting that these cells either lysed or reverted back to the normal blue-green fluorescence. We did not observe red fluorescence in cells

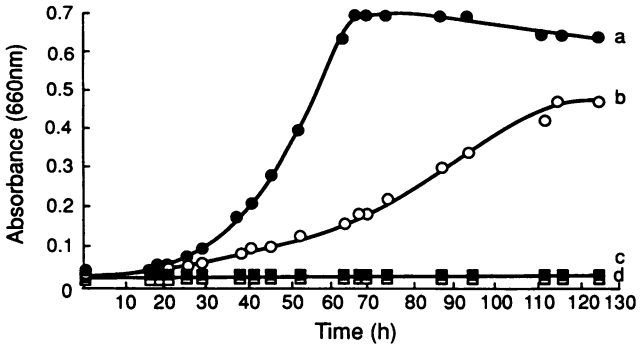

FIG. 3. Growth of *M. acetivorans* in the presence and absence of light. The curves are labeled as follows: a, growth of cultures that were maintained in the dark; b, growth of a culture in the presence of light that had passed through a 500-nm interference filter (the width of the transmission curve at 50% of the maximum transmission was 71 nm); c, growth of cultures in the presence of light that had passed through a 398-nm interference filter (the width of the transmission curve at 50% of the maximum transmission was 55 nm); and d, growth of cultures that were maintained in the presence of light (8 W m^{-2}, blue fluorescent).

grown in the presence of light that completely prevented growth or in cells grown completely in the dark. Rouvière and Wolfe have previously observed spontaneous red fluorescence of a *Methanosarcina* isolate (strain WH1) (13). The red fluorescence was not, however, a light-induced phenomenon.

We also passed blue light through a biological filter that contained purified coenzyme F420 in 50 mM KP_i (pH 9.0) (Fig. 4). The final A_{420} was 2.0 (path length = 1.5 mm). An identical filter that contained only 50 mM KP_i (pH 9.0) was made. Control tubes were wrapped in aluminum foil. All tubes were continuously illuminated with a blue fluorescent lamp (8 W m^{-2}). As seen in Fig. 5, light that passed through a filter jacket containing only buffer totally inhibited growth, whereas light that had passed through the filter containing coenzyme F420 showed only moderate growth compared with that in the control tubes. These data are consistent with those obtained with the interference filter. We also observed red fluorescence of *M. acetivorans* cells which were incubated in the F420 filter. We observed no red fluorescence with the culture incubated in the filter containing only buffer; that is, under conditions that completely inhibited growth.

DISCUSSION

Each of the methanogenic archaebacteria (archaea) examined was found to be light sensitive. Clearly, much needs to be learned about the absorbing species and the mechanism of light inhibition. There are at least two known chromophores found in methanogens which absorb light in this region: coenzyme F430, a novel nickel-containing corphin that absorbs maximally at 430 nm (11, 20), and coenzyme F420, a deazaflavin that absorbs maximally at 420 nm (4). Data obtained from the interference filters and the F420 biological filter suggest that the inhibitory wavelengths of light lie between 370 and 430 nm. We observed a loss of cell viability of *M. voltae* grown in the presence of light. It is of interest that exposure of whole cells to air causes an accumulation of adenylylated and guanylylated F420 molecules (5, 18). Perhaps light stress may induce a similar increase of these modified molecules (i.e., "alarmones"), but we have not examined this possibility.

We reproducibly observed red fluorescence of *M. acetivorans* grown under light conditions intense enough to moderately inhibit growth but not so intense as to totally inhibit growth. We observed no red fluorescence of cells grown in the dark. Up to 20% of the cells fluoresced brilliant red when grown in the presence of light that had passed through the 500-nm interference filter. The source of this red fluorescence has not yet been determined.

We propose that the results presented here may be useful for anaerobic enrichment studies. A blue light can be used to prevent the growth of methanogens in enrichment cultures if some other strict anaerobe is desired. Since methanogens play a fundamental role in the biological flow of carbon in the environment (21), it would be of interest to study what effect the possible specific, nonintrusive light

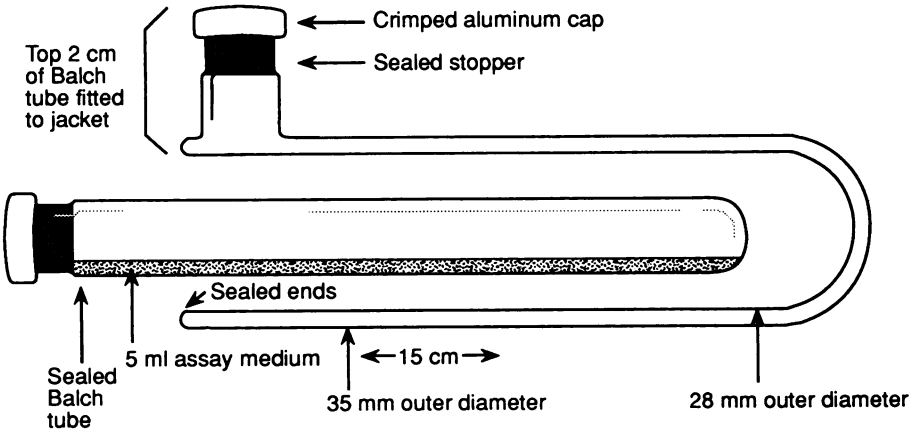

FIG. 4. Biological filter. The contents of the jacket were added in an anaerobic chamber. Two identical filters were made. One contained only buffer (50 mM KP_i, pH 9.0), and one contained purified coenzyme F420 in the same buffer. The final A_{420} was 2.0 (internal light path = 1.5 mm). A culture tube was placed in the jacket, and the entire filter was placed under a blue fluorescent light.

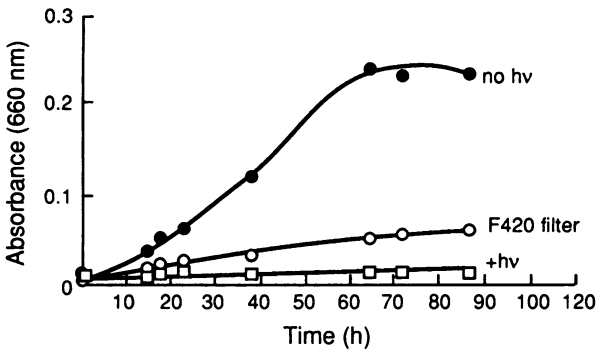

FIG. 5. Growth of *M. acetivorans* in the presence and absence of light. Control culture tubes were wrapped in aluminum foil. The growth curve labeled F420 filter refers to *M. acetivorans* grown in the presence of light that had passed through a jacket containing coenzyme F420 (A_{420} = 2.0; path length = 1.5 mm). The growth curve labeled +hν refers to *M. acetivorans* grown in the presence of light that had passed through a jacket containing buffer only.

inhibition of methanogens has on carbon flow in mixed populations of bacteria.

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