JOURNAL OF BACTERIOLOGY, Feb., 1966

Vol. 91, No. 2
Printed in U.S.A.

Action Spectrum for the Photolysis of *Myxococcus xanthus*

ROBERT P. BURCHARD, SOLON A. GORDON, AND MARTIN DWORKIN

Department of Microbiology, University of Minnesota, Minneapolis, Minnesota, and Argonne National Laboratory, Division of Biological and Medical Research, Argonne, Illinois

Received for publication 30 July 1965

During the course of an investigation of photolysis in *Myxococcus xanthus* (Burchard and Dworkin, J. Bacteriol. **91**:535, 1966), a preliminary crude action spectrum was determined by use of glass and gelatin filters to exclude broad areas of the visible spectrum. To attempt to identify the photosensitizer, a more refined relative spectral response was obtained with the Argonne National Laboratory biological spectrograph, described by Monk and Ehret (Radiation Res. **5**:88, 1956), and will be reported in this note.

The instrument's light source was a 14-kw carbon arc; the beam was diffracted to yield a linear dispersion of 1 A/mm on the spectral curve. Fresnel condensers were utilized to increase the irradiance to which the cells were exposed. Irradiancies were determined with an Eppley thermopile calibrated against a National Bureau of Standards lamp.

M. xanthus FB_t was cultured in low Mg-CT medium (Burchard and Dworkin, J. Bacteriol. **91**:535, 1966) in the dark on a rotary shaker at 30 C. Cells, approximately 36 hr into the stationary phase, were exposed to a band of light (1 mμ wide) at 10-mμ intervals between 350 and 700 mμ; 5-mμ intervals were used to define more precisely the spectral regions of lysis.

Prior to exposure to light, cells were diluted 1:20 in fresh low Mg-CT medium and distributed in 2.5-ml samples in 3-ml Pyrex cuvettes. The cuvettes were inserted into rigid holders at various wavelengths. During illumination, cells were aerated and agitated by bubbling washed, humidified air through each suspension.

Photolysis was assayed by periodic counts of surviving cells with a Petroff-Hausser counting chamber.

Five major experiments were carried out in spectral regions of photolysis; each experiment tested 5 to 10 wavelengths. Other apparently nonphotolytic wavelengths were studied in minor experiments.

As an example, the results of irradiation with five wavelengths run simultaneously in experiment 4 are presented in Fig. 1. The logarithm of surviving cells is plotted against radiant density. The points represent total cell counts and are best described by a "multi-hit" curve with the relationship $S = 1 - (1 - e^{-kD})^n$ (Powers, Phys. Med. Biol. 7:3, 1962), where S is the standardized response (fraction of survivors) to a dose D, and k is a constant, describing the slope of the curve; n, also a constant, has been interpreted as the number of "hits" necessary to bring about the death of cells exposed to ionizing radiation and, analogously, might here reflect the minimal number of photons required for lysis at a given wavelength.

The data were programmed in Fortran and analyzed on an International Business Machines model 1620 computer (Tyler and Dipert, Phys. Med. Biol. 7:201, 1962). Each curve in Fig. 1 represents the optimal fit for the experimental points obtained at each wavelength.

Table 1 presents the k and n values of photolysis at each wavelength for the five major experiments. Recalculation of k is accomplished by correcting the five $k_{425m\mu}$ values to the lowest value. In each experiment, all other wavelengths are corrected by the same factor that was required for the $k_{425m\mu}$ correction. This compensates for the variation of photosensitivity with age of cells among different experiments. A 375-mμ

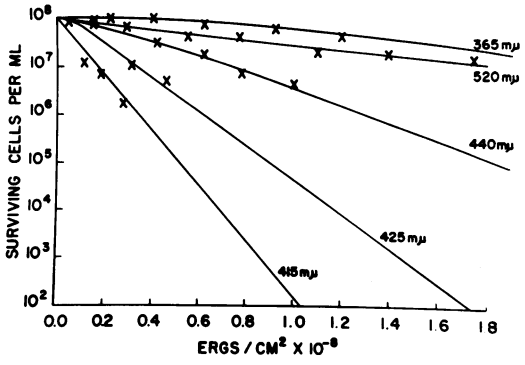

FIG. 1. *Photolysis of Myxococcus xanthus FB_t with monochromatic light.*

TABLE 1. *Slopes and "extrapolation" numbers for photolysis at various wavelengths (monochromatic light)*

Expt	Wavelength	k (slope)	n ("extrapolation" no.)	k (recalculated to standard 0.574×10^{-7} value for 425 mμ)
	mμ			
1	375	2.39×10^{-8}	2.39	0.16×10^{-7}
	385	6.12×10^{-8}	2.41	0.42×10^{-7}
	396	1.10×10^{-7}	2.04	0.74×10^{-7}
	410	4.56×10^{-9}	823	
	420	1.00×10^{-10}	823	
	432	3.66×10^{-8}	8.37	0.25×10^{-7}
2	375	1.91×10^{-8}	2.05	0.16×10^{-7}
	390	3.78×10^{-8}	1.95	0.33×10^{-7}
	410	1.79×10^{-7}	5.28	1.55×10^{-7}
	425	6.63×10^{-8}	8.25	0.57×10^{-7}
	435	4.76×10^{-8}	6.49	0.41×10^{-7}
3	370	5.26×10^{-8}	2.10	0.26×10^{-7}
	380	7.72×10^{-8}	7.18	0.38×10^{-7}
	405	3.30×10^{-7}	8.59	1.57×10^{-7}
	425	1.16×10^{-7}	1.21	0.57×10^{-7}
	500	1.07×10^{-8}	3.39	0.05×10^{-7}
	510	5.43×10^{-8}	5.63	0.27×10^{-7}
	525	7.22×10^{-8}	399	0.36×10^{-7}
4	365	1.63×10^{-8}	3.59	0.11×10^{-7}
	400	4.75×10^{-8}	0.58	0.33×10^{-7}
	415	1.28×10^{-7}	0.60	0.88×10^{-7}
	425	8.33×10^{-8}	1.98	0.57×10^{-7}
	440	4.20×10^{-8}	2.39	0.29×10^{-7}
	505	9.12×10^{-9}	0.55	0.06×10^{-7}
	520	9.95×10^{-9}	0.68	0.07×10^{-7}
	550	1.24×10^{-8}	1.29	0.08×10^{-7}
	560	2.42×10^{-8}	114	0.17×10^{-7}
	640	7.88×10^{-9}	3.53	0.05×10^{-7}
5	325	∞ (10^{-24})	1.00	0
	355	1.60×10^{-8}	3.82	0.16×10^{-7}
	425	5.74×10^{-8}	0.46	0.57×10^{-7}
	450	1.49×10^{-8}	3.35	0.15×10^{-7}
	490	1.08×10^{-8}	3.59	0.11×10^{-7}
	515	7.62×10^{-8}	8.60	0.76×10^{-7}
	535	3.11×10^{-8}	2.67	0.31×10^{-7}
	555	7.53×10^{-9}	2.49	0.08×10^{-7}
	600	1.31×10^{-8}	39.7	0.13×10^{-7}
	655	∞ (10^{-23})	1.00	0

standard was used in experiment 1, since 425 mμ could not be run simultaneously with 420 and 430 mμ.

The k value was used as a measurement of the efficacy of each wavelength in inducing photolysis. These values are plotted against wavelength in the lower part of Fig. 2, which depicts the photolysis action spectrum. High k values (high rate of lysis) are plotted as peaks on the action spectrum; zero k values are plotted as troughs. The points plotted in the upper portion of Fig. 2 give the cumulative energy delivered to the cuvette at the termination of the experiment. At wavelengths above 550 mμ, only high radiant densities elicited any response; these resulted in extended lag periods and low rates of photolysis. In contrast, photolysis at 410 mμ was essentially complete after a relatively low radiant density, with a virtually exponential survival curve. Other points showing no lysis or a low level lysis might be spurious, since it was difficult to attain high total energies at the longer wavelengths; the carbon arc had relatively low emission in this spectral region. With extended periods of irradiation, problems of cell clumping and evaporation of medium were encountered.

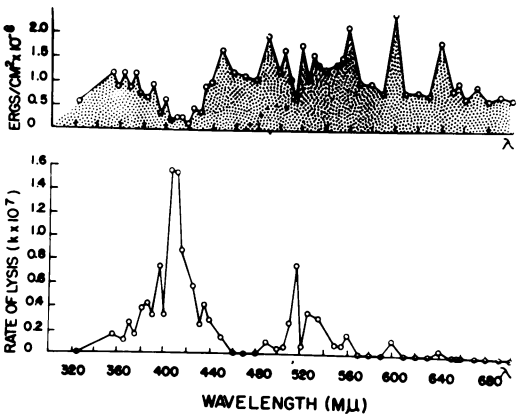

FIG. 2. *(A) Cumulative energy dosage at each wavelength. (B) Action spectrum of photolysis of Myxococcus xanthus FB$_t$.*

The action spectrum depicted in Fig. 2 has a striking similarity to the generalized absorption spectrum for porphyrins (Lascelles, *Tetrapyrrole biosynthesis and its regulation*, W. A. Benjamin, Inc., New York, 1964).

We gratefully acknowledge the technical assistance of Edward Barr with the spectrograph, and Merlin Dipert in data computation.

This investigation was supported by grant NSF GB-9 from the Developmental Biology Section of the National Science Foundation, and by Public Health Service training grant AI 90-05 from the National Institute of Allergy and Infectious Diseases. One of the authors, Martin Dworkin, is a Public Health Service Career Development Awardee, 1-K3-GM-5869-101.