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PHOTOMORPHOGENESIS IN PENICILLIUM ISARIIFORME I. THE ACTION SPECTRUM FOR LIGHT-INDUCED SPORULATION

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SUMMARY

The influence of light upon sporulation in *Penicillium isariiforme* was studied by determination of the action spectrum for this reaction. This action spectrum, with a single peak at 470–480 nm, differs strongly from action spectra obtained for other light-sensitive fungi. This may be caused by a different photoreceptor being present in this fungus or by a distortion by an unknown pigment.

1. INTRODUCTION

Penicillium isariiforme Stolk & Meyer, a fungus belonging to the Fungi imperfecti, shows a characteristic response to illumination. No information is available about the growth in the natural environment, but under laboratory conditions there is a great difference between growth in a light-dark regime and growth in total darkness. In the dark a flat yellow-coloured mycelium expanding in horizontal direction is formed. One short illumination with low-intensity light induces a rapid formation of conidiospores, whereas at higher light intensities the formation of coremia ensues. The coremia consist of a large number of hyphae; they show a very distinct positive phototropism. Because the coremia eventually will also sporulate, one might speculate about their possible significance for spore dissemination.

In the present study an attempt was made to obtain information about the nature of the photoreceptor mediating the light induced sporulation of hitherto vegetative mycelium. For this purpose action spectra were determined. In subsequent papers action spectra for the formation of coremia and for the phototropic reaction of the coremia will be given.

2. MATERIAL AND METHODS

2.1. Culture

Penicillium isariiforme Stolk & Meyer, strain 530, was obtained from the C.B.S. (Centraalbureau voor Schimmelcultures, Baarn, Holland).

The fungus was grown on a synthetic medium, containing 0.94 g NH_4NO_3 0.5 g KCl 0.5 g $MgC_3H_5(OH)_2PO_4$ 0.01 g $FeSO_4$ 0.35 g K_2SO_4 30.0 g sucrose 12.0 g ionagar in one litre (final volume) demineralized water. To this medium was added 1.0 ml of a micronutrient solution according to TAHA & KNIGHT (1961), consisting of 15.5 mg $CuCl_2$ 175.6 mg $ZnSO_4.7H_2O$36.0 mg

$MnCl_2.4H_2O$ 183.4 mg $CaCl_2.2H_2O$ 10.2 mg $(NH_4)_6Mo_7O_{24}.H_2O$ and demineralized water to 100 ml.

The fungus was cultivated in Petri dishes in which 30 ml of the medium was poured out. The dishes were placed in the dark in an incubator kept at 25 °C (± 1 °C).

2.2. Safelight

It was established in several preliminary experiments that weak red light from a Philips PF712 darkroom lamp did not affect growth and development of the fungus.

2.3. Inoculation

From special stock cultures a spore suspension in 50 ml sterile demineralized water was prepared. From this suspension kept in a refrigerator at about 2 °C a 1:25 diluted suspension was made. With a sterile pipette the petri dishes were inoculated in the center with one drop. This way of inoculation produced uniform cultures of the fungus.

2.4. Illumination

The light source used was a Leitz Prado 500 Watt slide proiector with special 400 mm objective in a construction modified according to MOHR & SCHOSER (1959) as illustrated in *fig. 1.* The light source was placed outside the room in which illumination of the fungi took place. The illumination time was controlled by means of the special shutter Shanel A5. The open shutter gives a lighted surface of about 5 cm diameter. Using this shutter has the advantage of exact

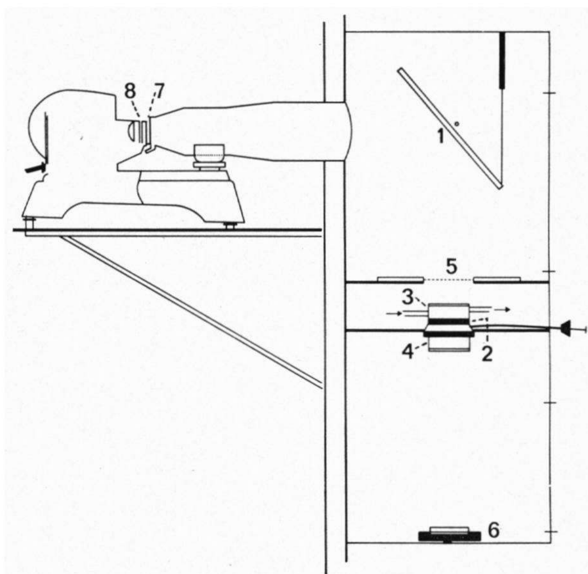

Fig. 1. Illumination apparatus used.
1. surface mirror
2. shutter
3. water filter
4. plexiglass filter
5. neutralglass filter
6. petri dish
7. interference filter
8. neutral glass filter

timing with a stopwatch at a constant light intensity. The electric current was stabilized with a Philips stabilizer PE 1002.

2.5. Filters

In the projector only interference filters were used: Filtraflex type B 20 from Balzers, Liechtenstein. Bandwidth 6–8 nm; wavelength tolerance 0,3%; size 50 × 50 mm. The filters gave a maximum transmission at the following wavelengths: 360 – 379 – 382 – 391 – 400 – 406 – 410 – 419 – 426 – 434 – 446 – 449 – 459 – 466 – 470 – 476 – 481 – 491 – 499 – 510 – 530 – 560 – 600 – 630 – 660 – 700 and 730 nm. With a combination of one or more neutral Schott filters, series NG 4, NG 3, NG 9 and NG 11, varying in thickness from 0.5 to 2.0 mm it was possible to get every desired light intensity without changing the intensity of the light source. The projection lamp, Osram, 110 Volt – 500 Watt no. 58880 E, was always used at 105 Volt. In this way the projector produced a constant light intensity during a rather long period. In order to get very low light intensities a sheet of 3 mm plexiglass (Röhm and Haas Darmstadt no. 800 or no. 838) was placed over the shutter.

2.6. Measurement of light intensity

Light intensity was measured by means of a compensated thermopile type CA 1; 1 Watt/m2 = 12,5 microvolt and a compensated thermopile type E5; 1 Watt/m2 = 45 microvolt. Recording of the measurements was performed with a Micrograph BD5. The whole equipment was obtained from Kipp en Zn., Delft, Holland.

Very low light intensities were measured with a photocell Philips O.R.P.63. This photocell was used in a device consisting of two stabilizers Philips PE 4808 and a microvolt meter Microva A.L.4 from Kipp en Zn., Delft; *fig. 2* and *fig. 3*. The changes in resistance in the O.R.P. 63 were calibrated for each wavelength with the aid of the very sensitive thermopile E 5. At light intensities below 60–

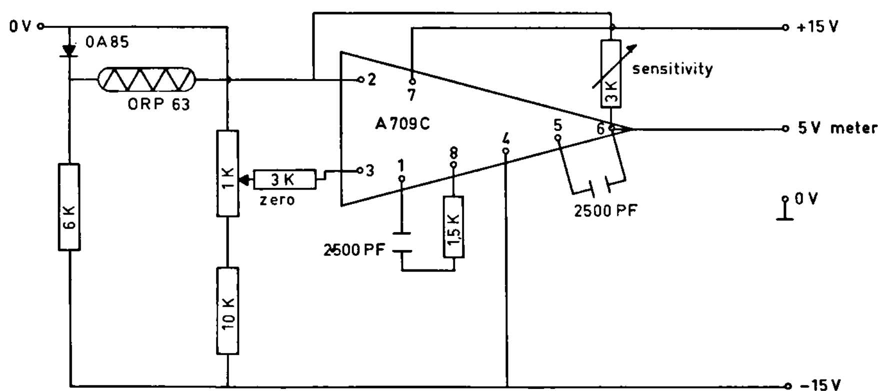

Fig. 2. Diagram amplifier A 709 c and photocell O.R.P. 63.

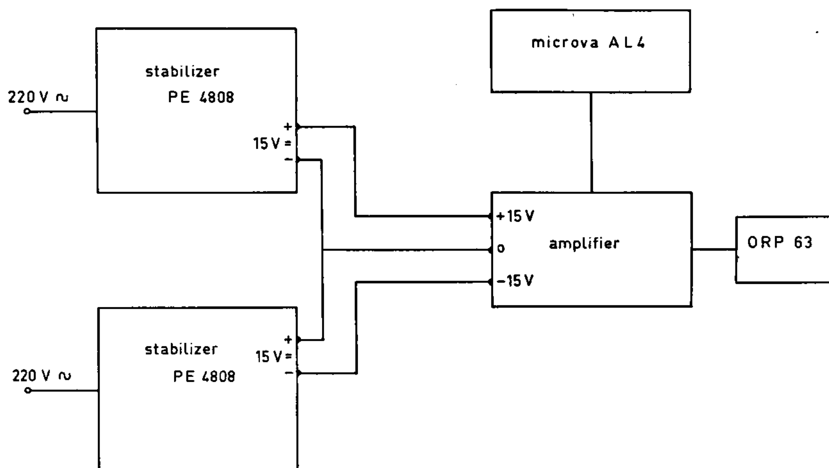

Fig. 3. Diagram amplifier A 709 c, photocell O.R.P. 63, stabilizer and microva AL 4.

100 erg.cm^{-2}.sec^{-1} the calibration curve for each filter appeared to be a straight line (*fig. 4*). For measurements of light intensities higher than 60–80 erg.cm^{-2}.sec^{-1} the thermopile CA 1 was used.

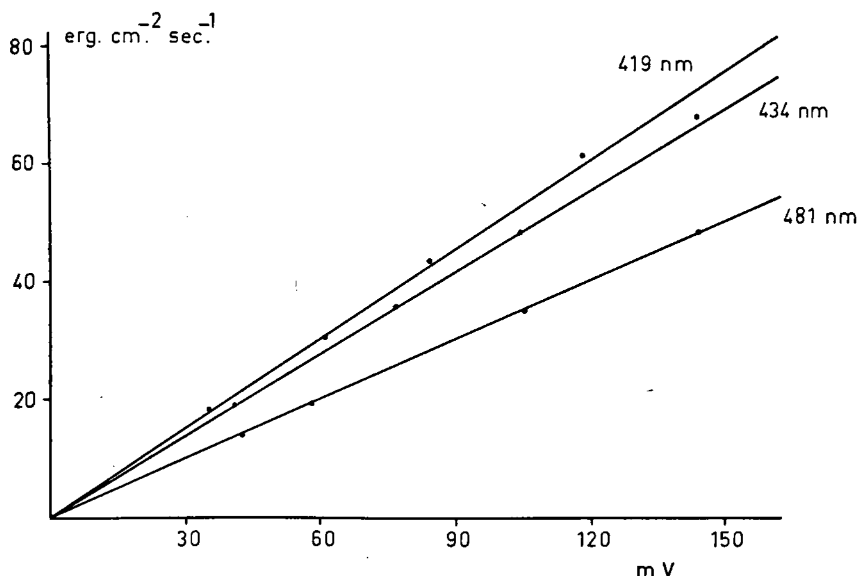

Fig. 4. Some examples of calibration curves for measurement of low light intensities.
Abscissa: values obtained with photocell and microva AL4.
Ordinate: values obtained with thermopile E5 and micrograph BD5.

3. RESULTS

Inoculations of *P. isariiforme* in Petri dishes with 30 ml medium were placed in a dark incubator during five days. After that time the mycelium had a diameter of about 20 mm. These cultures were illuminated with monochromatic light. Preliminary experiments showed that a very low light intensity during a few seconds gave a maximum sporulation.

It was impossible to establish dose–response–curves for this sporulation. When a certain minimum dose of light was given a maximum sporulation was produced by the mycelium. Apparently light-induced sporulation is an all-or-none response in this fungus. Therefore it was necessary to ascertain whether the Bunsen-Roscoe reciprocity-law holds true. This means that the product of light intensity (I) and minimum effective time of illumination (t) should be constant.

For light intensities ranging from 8.5 to 35 $erg.cm^{-2}.sec^{-1}$ this $I \times t$ relation appeared to be valid as is shown in *table 1* for three different wavelengths.

Table 1. $I \times t$ relation for the sporulation of *P. isariiforme*.

Wavelength nm	Intensity (I) $erg.cm^{-2}.sec^{-1}$	Minimum effective time of illumination (t)	$I \times t$
426	35	4–5 sec.	157.5
426	9	16–20 sec.	162
449	35	4–5 sec.	157.5
449	8.5	16–20 sec.	153
476	35	1 sec.	35
476	10	3–4 sec.	35

The light intensity transmitted by each filter was fixed at 35 $erg.cm^{-2}.sec^{-1}$ with the exception of filters 361 nm, 382 nm and 400 nm, which transmitted 10, 31 and 31 $erg.cm^{-2}.sec^{-1}$ respectively.

With each filter four to six experiments were performed in which the time of illumination was varied from 1 to 35 seconds. In every experiment always two parallel cultures were illuminated during the same period of time so that the limit for sporulation was determined for every filter 8 to 12 times. The variation in the different experiments was rather low, so that a reliable mean value could be determined for every filter as is shown in *table 2*.

The relative quantum efficiency in table 2 is calculated according to the following formulae:

$$1 \text{ quantum/sec} = \frac{1987}{\lambda} \times 10^{-18} \text{ Watt} \qquad (1)$$

$$1 \text{ Watt/m2} = 1000 \text{ erg.cm}^{-2}.\text{sec}^{-1} \qquad (2)$$

Substitution of (2) into (1) produces:

$$1 \text{ erg.cm}^{-2}.\text{sec}^{-1} = \frac{\lambda}{1987} \times 10^{11} \text{ quanta. cm}^{-2}.\text{sec}^{-1} \qquad (3)$$

Table 2. Sporulation in *P. isariiforme* in connection with wavelength and time of illumination

Wavelength nm	Intensity erg.cm^{-2}.sec^{-1}	Mean effective time for sporulation sec.*	Total energy erg.cm^{-2}	Rel. quantum efficiency
361	10	36.0 (–)	360	0.13
379	35	5.5 (0.7)	193	0.24
382	31	6.0 (0.5)	210	0.21
391	35	7.5 (0.8)	263	0.16
400	31	7.7 (1.1)	239	0.18
406	35	8.5 (0.5)	298	0.14
410	35	7.8 (0.8)	273	0.15
419	35	6.2 (0.5)	217	0.18
426	35	5.5 (0.5)	193	0.20
434	35	6.0 (0.8)	210	0.18
446	35	3.8 (0.8)	133	0.28
449	35	3.8 (0.6)	133	0.28
459	35	2.5 (0.5)	88	0.41
466	35	1.5 (0.5)	53	0.68
470	35	1.0 (0.4)	35	1.02
476	35	1.0 (0.0)	35	1.00
481	35	1.0 (0.4)	35	0.99
488	35	1.5 (0.5)	53	0.64
491	35	3.5 (0.5)	123	0.28
499	35	5.5 (1.3)	133	0.17
510	35	30.0 (–)	1050	0.03

* Standard deviation.

A plot of these values versus wavelengths yields the action spectrum for light-induced sporulation as shown in *fig. 5.*

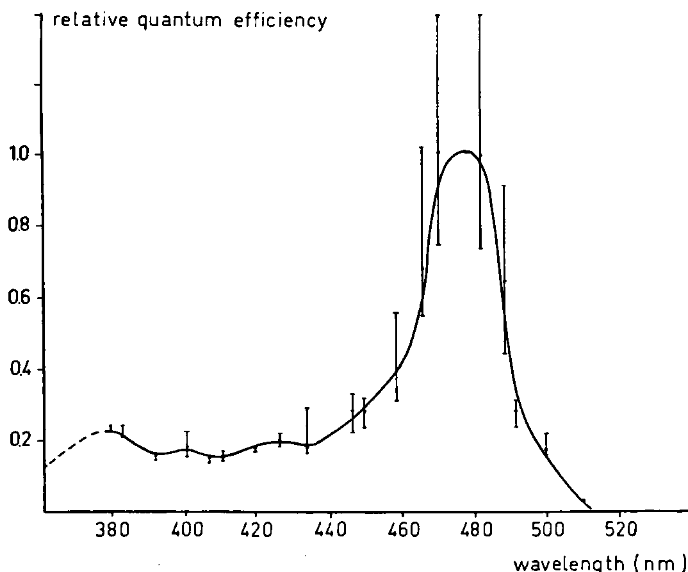

Fig. 5. Action spectrum for light-induced sporulation in *P. isariiforme.*

4. DISCUSSION

Action spectra for sporulation in fungi have been determined by several authors. In older experiments it was shown that light between 400 and 500 nm is most effective. Recently action spectra were given by GRESSEL & HARTMANN (1968) and KUMAGAI & ODA (1969) for *Trichoderma viride*. They found maximum effectiveness of light upon sporulation at 380 nm, at 430–440 nm and at 480 nm. At wavelengths over 520 nm there is no activity of light. The latter is in agreement with my findings.

Action spectra for other light reactions in fungi, e.g. phototropical reaction in *Phycomyces blakesleeanus*, show two maxima: at 380 nm and at 440–445 nm with a shoulder at 470–485 nm (DELBRÜCK & SHROPSHIRE 1960, CURRY & GRUEN 1959).

The action spectrum for *P. isariiforme* strongly differs from those published elsewhere, indicating that either in *P. isariiforme* the photoreceptor is different or the action spectrum is distorted by the presence of other pigments beside the photoreceptor. The latter is mentioned by DELBRÜCK & SHROPSHIRE (1960) for the area below 370 nm, where the phototropic response of the sporangiophore of *Phycomyces* is opposite to the reaction at higher wavelengths. The presence of certain pigments might be related to a kind of protecting or shielding function as suggested by KRINSKY (1968) for microorganisms.

The action spectrum for light-induced sporulation in *P. isariiforme* shows no similarity with any action spectrum to be found in literature. It gives no information about the photoreceptor; especially not as long as no information has been obtained about pigments present in the fungus.

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