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Antagonistic action of light in sporulation of the myxomycete *Physarum nudum*

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It has been found as a result of investigations on the action spectrum of the slime-mold *Physarum nudum* (Rakoczy 1963, 1965) that sporulation of this species was induced by 12 hours irradiation with light of two spectral ranges: 330—540 and 630—713 nm wavelengths, whereas the middle range of the spectrum from 540—620 nm, did not induce sporulation.

Lieth (1956) found that green light (he used a VG 9 filter) was not only deprived of activity in inducing sporulation of the myxomycete *Didymium nigripes*, but had an inhibitory action on this process. When cultures were irradiated with red light, of intensity sufficient to induce sporulation with a simultaneous supplement of green light of different intensities the percentage of sporulating plasmodia diminished. He found a close correlation between the percentage of sporulating plasmodia and the intensity of green light (the light was not a monochromatic radiation).

In view of Lieth's results it was considered necessary to make similar researches on *Physarum nudum* and to establish whether for this species the light in the range from 540—620 nm is simply deprived of effect on sporulation as it was in the case of *Didymium nigripes*, or on the contrary, this spectral range exerts an inhibitory effect on this process. Accordingly studies were made with the aim to obtain the relative activity in the inhibition of sporulation by individual wavelengths in the middle range of the visible spectrum.

MATERIAL AND METHODS

Plasmodia of the myxomycete *Physarum nudum* Macbr. cultured on 3% oats agar medium provided the experimental material. Cultures were kept in a dark thermostat at 21°C. The experimental procedure was similar to that described in an earlier paper (Rakoczy 1965). Plasmodia after 12 days culture in darkness were transferred on to a filter paper moistened with distilled water and placed in darkness until the moment when they regenerated and migrated on the surface of the filter paper. Then, small discs of the filter paper with fragments of a plasmodium were cut and placed in plexiglass vessels.

For irradiation of the plasmodia a special apparatus was devised. It was similar to that described earlier (Rakoczy 1965); the main difference being that each of

the light-proof chambers in which the plasmodia were irradiated was equipped not with one, but with two optical systems directing and concentrating the light beams emmited by the lamps.

For simultaneous irradiation of the plasmodia with two beams of light it was necessary to use two optical systems and two lamps as light sources. The first emmited blue light (BG 12 filter) which was able to induce 50% sporulation in the studied species; the second lamp was a source of light for obtaining radiations of different

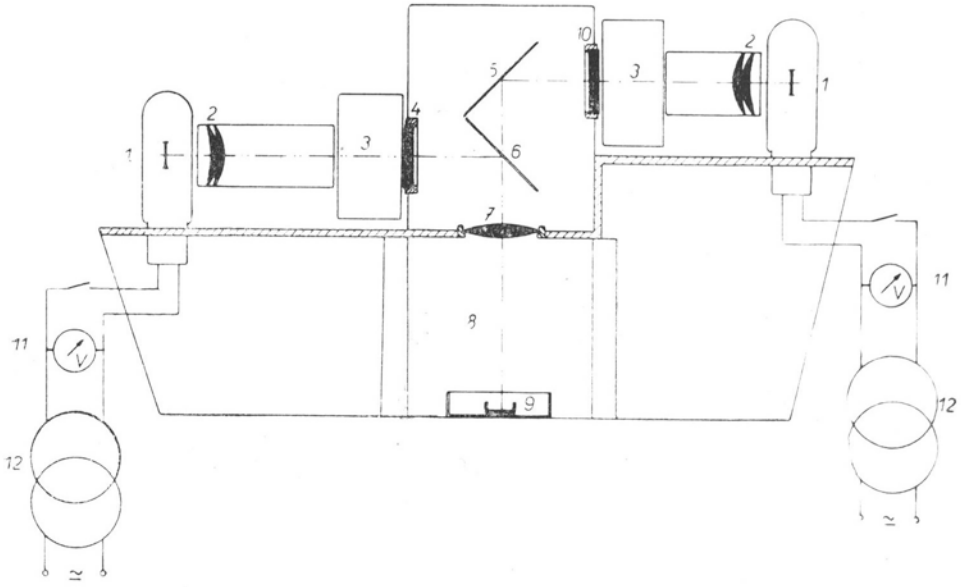

Fig. 1. Scheme of the apparatus used for irradiation.

1. Projector lamps 750 W; *2*. Condenser lenses; *3*. Water filter; *4*. Interference filter; *5*. Mirror; *6*. Semitransparent mirror; *7*. Collector lens; *8*. Light proof chamber with water coat; *9*. Plexiglass vessel with plasmodium; 10. BG 12 filter; *11*. Voltameter; *12*. Auto-transformer

wavelengths in the range 504—622 nm with the help of interference filters and different intensities. For this purpose it was necessary to use two mirrors for reflection of the light beams. The first was an usual one and the second was semitransparent (Fig. 1, 6). The two kinds of light were mixed and concentrated to form one beam illuminating uniformly an area about 12 mm of diameter. The apparatus consists of light proof chambers in which the plasmodia were irradiated. The walls of the chambers were surrounded with a water coat, which ensured a constant temperature of $21 \pm 1°C$ during the irradiation of the plasmodia. Each chamber was equipped with two similar optical systems with mirrors concentrating and directing the light beams downwards to the chamber. Each optical system included two condenser lenses ($2 \times 10D$) a water thermofilter, 5 cm thick, a colour filter, a mirror and one collector lens ($+10D$) common for both systems. The only difference between the two optical systems was that in the first system interference filters and a usual mirror

were used, and in the other a BG 12 filter and a semitransparent mirror, which transmitted about 50% of the taken light were applied.

As light sources 750 W, 110 V projector lamps were used. The lamps were fed with the help of an auto-transformer controlling the voltage. Fig. 1 presents the scheme of the apparatus used for irradiation. Zeiss (Jena) and Schott (Mainz) interference filters were used with the following maxima: 504, 520, 541, 557, 581, 602, 612, 622 and 669 nm wavelength, and BG 12 Schott filter (2 mm thick) for blue light. The maximum transmission of the interference filters ranged from 25—35% and the half width of the transmitted radiation equalled 6—12 nm.

The measurement of light intensities was carried out by means of a photocell (Lange) which was calibrated in erg/cm²sec for each wavelength by comparing its readings with the readings of a thermopile (Kipp and Zonnen) of a known absolute sensitivity. All the procedures related to the measurement of the light intensities were carried out in the same way as this was done during the research on the action of monochromatic light on sporulation (Rakoczy 1963, 1965).

Plasmodia were irradiated for 12 hours. After irradiation they were transferred to a dark thermostat in which sporangia (if formed) appeard within about 12—24 hours following irradiation.

Results were recorded in terms of percentage of sporulating plasmodia for each wavelength and each light intensity. The intensity of the blue light was constant and was sufficient to induce 50% sporulation of the irradiated plasmodia. The experiments were run in 10—15 replications. All the preparatory operations were carried out in the very weak day light just before experiments.

The aim of the present work was to check whether the studied wavelengths exert an antagonistic effect with respect to blue light and if so how great the relative activity of the radiation in question is. It is expected that in case of an inhibitory action the percentage of sporulating cultures (which is 50% in blue light only) will decrease or that the plasmodia will not sporulate at all.

RESULTS

Plasmodia irradiated with blue light whose intensity was sufficient to induce 50% sporulation of the irradiated cultures and simultaneously illuminated with accesory light showed differences in their ability to sporulate which appeared to depend on the wavelength and the intensity of accesory light.

In plasmodia irradiated with blue light and with light 504 nm wavelength the action of the two kinds of light is synergic and the percentage of the sporulating plasmodia increases concomitantly with the increase of the 504 nm light intensity. Similar results were obtained after irradiation of the plasmodia with light of 520 nm wavelength. However, after irradiation of the plasmodia with blue light and at the same time with 541 nm wavelength as accesory light the percent of sporulating plasmodia increased concomitantly with the increase the light intensity, but to a certain extent only. If the intensity of the accesory light is still increased the percentage of sporulating plasmodia decreases from 50 to a lesser rate.

The action of light of 557, 581 and 602 nm wavelength is distinctly inhibitory and in no case did the % of sporulation exceed 50. For example for light of 557 nm wavelength the intensity 1540 erg/cm² sec decreased the percent of sporulation from 50 to 40% of the irradiated cultures, and 6700 erg/cm²sec permitted only 10% of the irradiated cultures to sporulate. Radiation corresponding to 581 and 602 nm wavelengths exerted a similar but less inhibitory action on sporulation. For 581 nm wavelength — 4600 erg/cm²sec — the percent of sporulation decrease to 33%, and for 16000 erg/cm²sec only 10%. For 602 nm — about 16000 erg/cm²sec gives only 30% of sporulating plasmodia. In the case of 612 and 622 nm wavelengths of the accesory, light, the highest intensity obtainable in our apparatus did not modify the normal percentage of the sporulating plasmodia i.e. 50%.

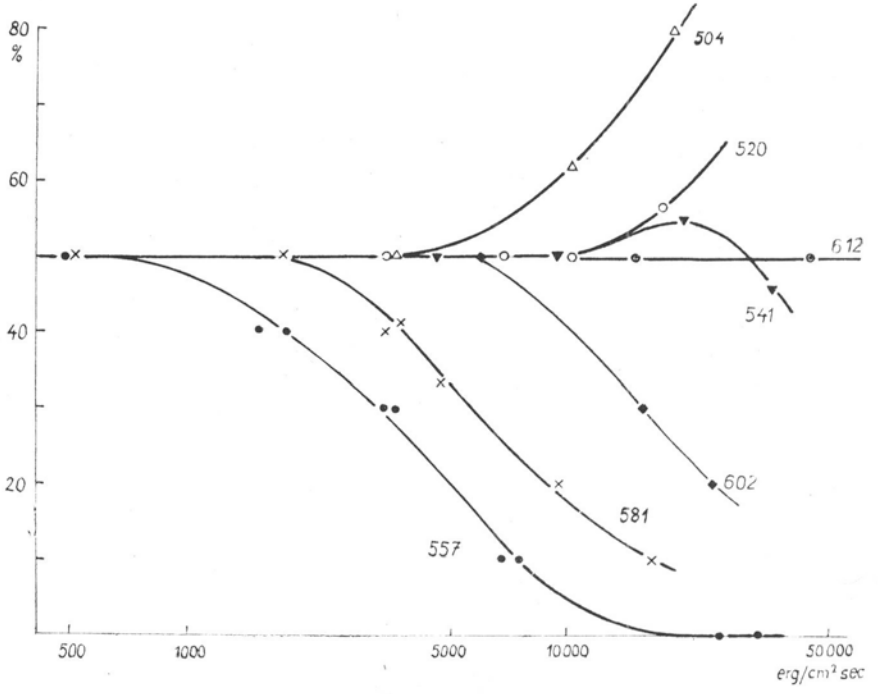

Fig. 2. Dependence of percent of sporulating plasmodia irradiated for 12 hours with blue and monochromatic light in the range 504—612 nm. on the intensity of the monochromatic radiation.

These results are illustrated in Fig. 2. Each curve represents, for a given wavelength, the results in dependence on the intensity of the accesory light (the intensity of the blue light was constant).

Light of the following wavelength: 541, 557, 581 and 602 nm exerts an inhibiting action on the percentage of the sporulating plasmodia; the 612 and 622 nm wavelengths are deprived of activity.

The interference filters used in these experiments made possible to compare the activity of individual wavelengths (541, 557, 581 and 602 nm) in their capacity to inhibit the sporulation process.

The radiation intensity (expressed in quanta/cm²sec) which decreases the percent of sporulating plasmodia from 50 to 25% was adopted as the criterion of inhibition. This intensity was found by graphical interpolation. The radiation of 557 nm wavelength was adopted as a point of reference.

The relative activity of the wavelengths inhibiting the sporulation is presented in Table 1 (the fourth column).

Table 1

Inhibitory influence of radiation on the sporulation process of the myxomycete *Physarum nudum*

Wavelength in nm	Intensity of radiation decreasing by 25% the standard percent of sporulation		Relative activity
	in erg/cm²sec	in quanta/cm²sec x 10^{13}	
(541)	(89200)	(2416)	(0.047)
557	4080	113.7	1
581	7080	206	0.552
602	20000	604	0.188

The 557 nm wavelength has the highest inhibitory activity in the sporulation process of the myxomycete *Physarum nudum* in relation to other studied wavelengths.

DISCUSSION

The results of the present research support the finding made by Lieth (1956) that green light exerts an inhibitory effect on sporulation when supplied simultaneously with light of other wavelengths of stimulatory activity. In the study of Lieth, however the spectral range of the green light was rather broad.

In the present study we have applied interference filters of narrow transmission which made possible to estimate the inhibitory activity for several wavelengths included in the spectral range in question. It become thus possible to obtain an action spectrum of the inhibitory activity of green light and to show that the maximum of the inhibitory effect corresponds to the wavelength 557 nm.

In the present experiments the inhibitory action of green light was studied with respect to the stimulating action of blue light.

Lieth (1956) made a study of the same effect with the respect to red light.

Additionally the action of radiation of 557 nm wavelength with respect to 669 nm (red light) was examined. The constant intensity of the red radiation was so high as to induce 50% sporulation in the irradiated cultures; the intensity, however, of the accesory green light (557 nm) was modified. It was found that in the case

the light of 557 nm wavelength had an inhibitory action with respect to red. This is in perfect agrrement with Lieth's results for *Didymium nigripes.*

On the ground of the results of both researches it may supposed that the antagonistic action of green light takes place against the stimulating activity independently on the wavelength of the latest one. The antaginistic effect of the middle part of the spectrum suggests the presence of two photoreceptor systems in the plasmodia controlling the processes of sporulation. One of them is responsible for the induction of sporulation. Its maximum absorption lies in UV, and probably has a smaller peak in red light. However, it can not be excluded that two different pigments, one absorbing the UV and the other red light, cooperate in the stimulating processes. The action of the other system is inhibiting the sporulation and antagonistic with respect to the activity of the first system.

Unsufficient knowledge of the plasmodial pigments and their absorption properties does not allow to make at the moment any suppositions about the pigments active as photoreceptors in the first and the second system.

I wish to express my most sincere thanks to Professor dr F. Górski and Professor dr J. Zurzycki for their valuable remarks in the course of this work.

SUMMARY

The range of the spectrum from 504—622 nm. wavelength has been studied with respect to its influence on sporulation of the myxomycete *Physarum nudum.*

The experimental material (12 days old plasmodia) was irradiated with two kinds of light simultaneously i.e. blue light inducing sporulation of 50% irradiated plasmodia and with monochromatic light of various wavelengths within the mentioned range and various intensities. BG 12 filter and interference filters were used for blue and monochromatic light respectively.

It has been found that the radiations of the wavelength from 540—602 nm. acted antagonistic in respect to the stimulatory action of blue light.

The relative activity of the individual wavelength active in inhibition of the sporulation process has been studied and it has been found that the highest inhibitory action is caused by the 557 nm. wavelength.

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Antagonistyczne działanie światła w procesie zarodnikowania śluzowca Physarum nudum

Streszczenie

Zbadano wpływ promieniowania środkowej części widma o długości fali od 504—622 nm na proces zarodnikowania śluzowca *Physarum nudum*. Plazmodia badanego śluzowca naświetlano równocześnie dwoma rodzajami światła: światłem niebieskim czynnym w procesie zarodnikowania o stałej intensywności dostatecznej dla wyzwolenia owocowania 50% naświetlanych kultur i światłem monochromatycznym o różnych długościach fali i różnych intensywnościach. Stwierdzono różny wpływ promieniowania na zarodnikowanie badanego śluzowca zależnie od długości fali i intensywności światła. O ile kultury były naświetlane światłem niebieskim o stałej intensywności (wyzwalającej owocowanie 50% kultur) i światłem o długości fali 504 lub 520 nm uzyskiwano wyraźne działanie synergistyczne obydwu rodzajów światła objawiające się zwiększeniem procentu owocujących kultur. Natomiast promieniowanie o długości fali: 541, 557, 581 i 602 nm ma wyraźnie hamujący wpływ na proces zarodnikowania co wyraża się wybitnym zmniejszeniem procentu kultur zdolnych do zaowocowania, o ile kultury te naświetlano równocześnie światłem niebieskim i promieniowaniem o wymienionych długościach fali. Efekt hamowania procesu zarodnikowania jest zależny od długości fali i intensywności promieniowania.

Z porównania względnej intensywności poszczególnych długości fal wpływającej hamująco na proces owocowania wynika, że najsilniejszy hamujący wpływ na ten proces wywiera promieniowanie o długości fali 557 nm. Promieniowanie o długościach fali 612 i 622 nm jest pozbawione aktywności.