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Biochemiluminescence of certain fungi

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Twelve species of fungi growing on the Sabouraud medium in darkness and illumination in an incubator, were tested to find out their ability to emit the ultra-weak biochemiluminescence. Using a sensitive photon-counting device, it was possible to measure biochemiluminescence intensity during ten days of cultures growth. *Boletus edulis, Pestalotia funerea* and *Microsporum gypseum* displayed biochemiluminescence, while *Aspergillus nidulans, A. quadrilineatus, Beauveria bassiana, Macrophoma candollei, Mucor lausanensis, Paecilomyces farinosus, Penicillium* sp., *Trichoderma lignorum* and *Tricholoma equestre* failed to do it. Illumination put down biochemiluminescence and stimulated colour formation in both mycelia and in the medium.

INTRODUCTION

Ultra-weak luminescence in the visible range of the spectrum, also called a biochemiluminescence (BCL) or biological chemiluminescence, occurs prevalently among higher plants and animals. There are relatively a few publications dealing with BCL of lower plants, particularly of fungi. Mamedov, Popov and Koniev (1969) studied over 80 species belonging to various systematic groups, inculding 9 species of fungi to find out their ability to give off BCL. They recorded a very weak luminescence of the order 30-50 photons per sec. per cm^2, emitted from *Saccharomyces, Schizosaccharomyces* and *Condida guillermondi.* Five other species: *Aspergilus glaucus, A. niger, Oidium lactis, Penicillium chrysogenum* and *Endomyces magnusi* did not reveal any measurable BCL.

Sławiński, Grabikowski and Murkowski (1971) disclo-

sed a clear BCL from *Agaricus bispora* and *Mucor* sp. while *Trichoderma lignorum* failed to exhibit luminescence being measurable by means of the apparatus used.

It is worthwhile mentioning that among fungi there are some species displaying three kinds of chemiluminescence, namely: strong bioluminescence involving the enzymic oxidation of $NADH_2$ ($NADPH_2$) e.g. fungi of the genus *Mycena*, *Collybia* and *Armillaria* (A i r t h 1961). micro--ultra-chemiluminescence, i.e. mitogenetic radiation which occurs during the budding of yeasts (*Saccharomyces*) and ultra-weak biochemiluminescence constituting the objective of the actual work. Fungi might be a convenient objective to study BCL because of their intensive metabolism and the fact that many species known to parasitize plants and animals can be easily cultivated on synthetic media.

The work presents the results of preliminary investigations on biochemiluminescence of some fungi species being cultivated on the Sabouraud medium in the darkness and in the light.

MATERIALS AND METHODS

Following species of fungi were investigated: *Aspergillus nidulans* (Eidam) Winter, *A. quadrilineatus* Thom et Raper, *Beauveria bassiana* (Balsamo) Vuil., *Boletus edulis* Bull. ex Fr., *Macrophoma candollei* (Berk. et Br.) Derl. et Vogl., *Microsporum gypseum* Bodin, *Mucor lausanensis* Lendner, *Paecilomyces farinosus* (Dicks ex Fr.) Brown et Smith, *Penicillium sp.* (group of *Asymetrica*), *Pestalotia funerea* Desm., *Trichoderma lignorum* (Tode) Harz and *Tricholoma equestre* Fr.

Above mentioned fungi were isolated and identified by workers of Plant Protection Department, Agriculture Academy in Szczecin.

Beauveria bassiana and *Paecilomyces farinosus* (*Deuteromycetes*) are entomogenous fungi. Both species were isolated from imago of *Leptinotarsa decemlineata* Say. *Microsporum gypseum* is a parasitic fungus living on human skin and hairs. Moreover, it is often found as a saprophytic species in the soil. *Macrophoma candollei* is a circumstantial parasite of plants. This species was isolated from *Buxus sempervirens* L. (M a d e j 1974). *Boletus edulis* and *Tricholoma equestre* (*Basidiomycetes*) are mycorrhizic fungi. The pure culture of mycelium were obtained from very young fruit bodies of *B. edulis* and *T. equestre*. Each single head was superficially sterilized in the alcoholic solution of 0,1% mercuric chloride and washed several times with sterile water. Then, a piece of tissue from inside of sterilized head was transferred to the Sabouraud medium under sterile conditions. The mycelium of both species grew

very slowly until they were inoculated the third time and subsequently their growth proceeded at a rapid rate.

The remaining species of the fungi mentioned above exist as saprophytes in the soil. All species grew well on the Sabouraud medium (15 g of agar, 10 g of peptone and 40 g of dextrose in 1 l of water).

30 ml of sterile Sabouraud medium was added to sterile Petri dishes 10 cm in diameter. After 24 h 1 ml of the suspension containing spores and or mycelium fragments was introduced into each of the dishes. In order to prepare suspension the pure cultures of fungi were cultivated on the Sabouraud medium in Petri dishes. After 6 days, a disc 2 cm in diameter was cut out, transfered to a Waring Blender and homogenized with 200 ml of sterile water during 5 min. Later on 1 ml of the homogenate was transfered into a Petri dish with the Sabouraud medium, the whole surface of the medium was equally covered with suspension and the growth of all species of fungi proceeded equally throughtout the surface of the medium.

The cultures were incubated at 26-28°C in 4 parallel repetitions. Petri dishes, containing only the medium without fungi, were used as controls. Both control and test dishes were placed in steril compartments — one being dark and the second one illuminated (220 lx) with fluorescen "daylight".

The first measurements started 24 h after the inoculation. Illuminated dishes were kept in complete darkness for 1 h to eliminate the phosphorescence of a glass, medium and mycelium.

MEASUREMENTS OF THE BIOCHEMILUMINESCENCE INTENSITY

Since intensity of BCL was very weak, a sensitive photoelectric apparatus employing the single photoelectron counting method had to be used (Sławiński, Grabikowski, Murkowski 1971). The S12FS52 Zeiss photomultiplier with spectral sensitivity 300-650 mm, cooled down to 200 °K with a solid CO_2 was applied as a detector of photons emitted by the fungi cultures. A signal from the photomultiplier output was 1000 times amplified and recorded by means of electronic counter PT-2 "Polon".

The measurements were carried out by determining the time (t) in the range of which the same number of counts (N) was recorded. A set number N was chosen in such a way that the value of t obeyed the condition: $90\ s > t > 20\ s$. Most frequently the N value was 4000. Measurements of t (N) were repeated 4-6 times for each Petri dish. During the measurements an emission level from the camera (a background) and from

the control Petri dishes was carefully and frequently checked. The temperature within the camera was constantly kept at 295 °K.

The results of measurements were subjected to the statistical analysis (Szepke 1967) using three magnitudes:

I_t — an average intensity of the background, i. e. a dark current of the photomultiplier and an emission from the camera's interior without a Petri dish,

I_k — an average intensity of emission from a control Petri dish i. e a chemiluminescence from Sabouraud medium and a glass phosphorescence of a Petri dish,

I_c — an average, total emission intensity i. e.

$$I_c = I_t + I_p + I_k$$

where I_p is an average BCL intensity from a test Petri dish. Since the intensity of the background was relatively constant ($I_k = 58.2 \pm 3.5$ s for $N = 4 \cdot 10^3$ counts i. e. 68.7 ±4.1 counts per s), therefore the sum $I_t + I_k = I_o$ was taken for calculations. Computations were performed by resorting to the following formula:

$$A = \frac{t_o - t_c}{t_c} \cdot 100\%$$

where t_o and t_c are the average counting time of a set impulses number N of the background and the control ($I_t + I_k$) and a total emission (I_c), A is a relative increase in impulses number for a test Petri dish over impulses number from a control and a background, expressed in percentage.

The obtained results were subjected to the statistical analysis according to Szepke (1967).

Avaluation of the Petri dish — photocathode geometry used at maximal efficiency of the photomultipier predicted the collection of approximately 2% of the total photons emitted. Calculations of the absolute BCL intensity give values of the order of 50-100 quanta$\times$cm$^{-2} \cdot$ s^{-1}.

RESULTS AND DISCUSSION

An analysis of the obtained results, presented in the Tables 1-3, provides the possibility to discus three paramount problems, namely: the relation between a relative BCL intensity (A value) and the species of fungi. the BCL kinetics during the cultivation of fungi, and the influence of ilumination on the BCL intensity and kinetics of particular species of the investigated fungi.

The most strongly emitting species appeared to be *Boletus edulis, Pestalotia funerea, Microsporum gypseum* and *Trichoderma lignorum*. The

Table 1

Relative values of biochemiluminescence intensity of fungi growing in darknes (A-values in %)

Fungus	Days of growing							Mean value
	1	2	3	4	5	6	7	
Aspergillus nidulans	−12.63 ± 2.19	−32.63 ± 6.00	16.53 ± 3.52	−9.22 ± 2.25	−16.37 ± 3.13	−5.04 ± 4.49	−7.95 ± 1.92	− 9.61
Aspergillus quadrilineatus	−21.51 ± 2.83	−32.95 ± 3.16	2.92 ± 2.08	−17.75 ± 3.47	−17.63 ± 2.19	−10.74 ± 4.08	−13.66 ± 1.89	−15.90
Beauveria bassiana	−6.17 ± 2.44	−27.65 ± 5.60	13.46 ± 3.40	−4.55 ± 2.48	2.96 ± 1.83	1.73 ± 2.33	3.58 ± 1.70	− 2.38
Boletus edulis	−12.68 ± 1.47	1.08 ± 5.84	77.96 ± 3.52	110.00 ± 1.92	105.91 ± 2.01	63.54 ± 1.71	64.18 ± 1.24	58.57
Marophoma candollei	−9.29 ± 2.19	−30.97 ±	21.47 ± 3.38	−33.54 ± 2.62	−25.49 ± 4.51	−16.78 ± 2.26	−16.42 ± 1.44	−15.86
Microsporum gypseum	17.22 ± 2.05	11.59 ± 5.55	127.74 ± 2.78	101.92 ± 2.62	28.52 ± 2.41	25.85 ± 1.79	7.00 ± 5.29	45.70
Mucor lausanensis	0.95 ± 2.59	−9.41 ± 5.80	13.14 ± 1.88	12.10 ± 2.19	−4.01 ± 1.93	1.88 ± 3.30	3.95 ± 1.72	2.66
Paecilomyces farinosus	−10.27 ± 1.50	−24.15 ± 3.60	33.71 ± 1.97	0.86 ± 1.94	−14.51 ± 2.75	17.27 ± 3.20	30.79 ± 1.84	4.38
Penicillium sp.	−27.60 ± 1.40	10.49 ± 3.32	−9.05 ± 1.50	−23.59 ± 2.25	−21.11 ± 1.09	−17.05 ± 1.97	−16.57 ± 1.20	−14.93
Pestalotia funerea	72.98 ± 1.92	7.48 ± 5.19	109.90 ± 3.14	52.50 ± 2.06	47.98 ± 0.70	38.94 ± 1.68	34.59 ± 1.30	52.01
Trichoderma lignorum	37.76 ± 1.92	−6.17 ± 3.49	86.77 ± 2.03	1.94 ± 2.19	0.29 ± 2.50	−4.93 ± 0.94	−12.97 ± 1.26	14.67
Tricholoma equestre	−9.20 ± 1.49	−14.11 ± 6.16	−7.92 ± 3.08	−29.37 ± 3.72	−31.39 ± 0.50	−19.35 ± 1.94	−18.34 ± 1.44	−18.52

intensity of BCL for three species cultured in darkness, is by 45-60% greater than the intensity of the emission from Sabouraud medium (a control). Differences between the I_c and I_t+I_k values are significant, because the values of the confidence intervals σ are lesser than $\pm 6\%$. *Paecilomyces farinosus* and *Mucor lausanensis* exhibit much weaker BCL and strong variations in BCL intensity during the incubation. BCL intensity for 6 remaining species is markedly lower than the emission intensity (I_k) from the Sabouraud medium. Relative changes of BCL intensity are most frequently negative ($A < 0$). The greatest decrease (—A) reaching up to 30% has been observed for the species *Tricholoma equestre*, *Aspergillus quadrilineatus*, *Macrophoma candollei* and *Penicillium sp.* It means that not only do these species give BCL intensity lower than that of Sabouraud medium, but they also quench its luminescence. In such a case the value of I_t+I_k is higher than I_p and values A are negative.

The kinetics of BCL of fungi cultures, i. e. the function $A=f(\tau)$ where τ is the time of incubation, displays considerable variations. In some cases that findings makes it very difficult to determine clear tendencies implied in the BCL intensity.

Generally, it can be concluded that the majority of the investigated species give the weakest BCL in the second day, and the strongest one on the third day of culturing in darkness. On the fourth and next days BCL intensity reveals distinctly lover variations of intensity and generally the level of BCL is lower. Individual differentiation of the kinetics is also observed. Thus, for example, *Boletus edulis* gives the strongest BCL between the third and fourth day of the incubation, while *Pestalotia funerea* and *Microsporum gypseum* reach the maximum of their BCL intensity in the second day of growing.

As it is evident from the comparison of data (Tables 1, 2) the effect of relatively weak illumination 220 lx upon the BCL intensity of fungi is distinctly negative. In all cases, excluding *Macrophoma candollei*, the illumination diminishes BCL intensity by 3-34% as compared with the cultures in darkness. The illumination does not influence the chemiluminescence of the Sabouraud medium since the ratio $(I_k/I_t)_{darkness}$ is 1.65, $(I_k/I_t)_{illum}$ is 1.62 and the intensity of a background $I_t=57.5\div68.3$ counts per sec. Differences between both values are statistically insignificant. The effect of light exerted on fungi growth was investigated by Łazarski (1971). He observed a reversible growth inhibition of *Aspergillus giganteus* Wehml. var. *alba* Zurz.

The observed effect of illumination cannot be explained exclusively by the possible decrease in mycelium metabolism and BCL connected

Table 2

Relative values of biochemiluminescence intensity of fungi growing under illumination (A-values in %)

Fungus	Days of growing						Mean value
	2	3	4	5	6	7	
Aspergillus nidulans	−38.05 ± 9.58	−40.74 ± 1.28	−23.64 ± 2.06	−7.08 ± 3.50	−21.48 ± 3.00	−34.69 ± 3.05	−27.61
Aspergillus quadrilineatus	−28.27 ± 9.12	−19.05 ± 1.13	−23.98 ± 1.72	−7.87 ± 3.33	−23.17 ± 2.12	−17.96 ± 2.90	−20.05
Beauveria bassiana	−19.67 ± 11.96	−18.67 ± 2.00	−25.07 ± 1.17	1.17 ± 0.92	−13.88 ± 2.41	−29.87 ± 1.56	−17.66
Boletus edulis	−14.35 ± 3.98	−4.52 ± 2.97	−11.57 ± 1.28	−8.31 ± 3.18	11.71 ± 2.41	6.91 ± 1.30	−3.35
Macrophoma candollei	−39.14 ± 10.38	−6.19 ± 3.49	−10.16 ± 2.69	8.43 ± 4.03	−0.25 ± 3.33	−15.73 ± 3.05	−10.51
Microsporum gypseum	−7.84 ± 5.28	−27.60 ± 0.80	−29.54 ± 1.44	11.02 ± 3.05	30.97 ± 3.33	11.30 ± 4.46	−1.95
Mucor lausanensis	−17.64 ± 5.60	−14.21 ± 3.60	−7.48 ± 2.72	−15.43 ± 3.96	−15.10 ± 2.41	−1.91 ± 3.60	−11.96
Paecilomyces farinosus	−17.49 ± 7.21	−16.05 ± 1.13	−17.04 ± 1.22	−5.46 ± 3.96	−10.80 ± 2.12	14.71 ± 2.50	−8.69
Penicillium sp.	−30.60 ± 7.40	−27.27 ± 7.30	−25.28 ± 4.27	−23.89 ± 4.23	−25.50 ± 3.33	−18.80 ± 1.65	−25.22
Pestalotia funerea	—	−3.58 ± 3.40	−9.24 ± 1.22	−17.17 ± 2.87	3.71 ± 2.69	−2.11 ± 2.33	−4.73
Trichoderma lignorum	19.54 ± 7.44	−35.09 ± 1.80	−31.43 ± 2.32	−21.27 ± 3.33	−27.41 ± 1.83	−20.28 ± 2.33	−19.32
Tricholoma equestre	−34.62 ± 2.42	−45.49 ± 3.94	−38.75 ± 1.22	−30.07 ± 1.22	−24.22 ± 2.11	−29.98 ± 1.70	−33.85

with it. In such a case the following condition should be obeyed: $I_k + I_t \leqslant I_c$
while, as a matter of fact, the reverse relationship is observed. The comparison of relative BCL intensity with observations of mycelium and medium colour provides essential information on the interpretation of negative values A and the illumination effect. Values of the average BCL intensity and the colours of mycelium as well as medium, observed on the third and fifth day of incubation, for several most strongly and most weakly luminescenting species are listed in Table 3. It is seen that strongly luminescent species have a colourless or almost white colour of the colony. The revers side of the colony on Sabouraud medium was cream-coloured, lemon-coloured or bright-yellow. The fungi exhibiting negative A values, had a brown, green or green-blue colour of mycelium. The medium was cherry, green-rust or rust-coloured with exogenous substances produced by mycelium.

The exact interpretation of the relation between the BCL intensity and the colour of mycelium is impossible because the spectral distribution of BCL is unknown. It seems, however, that radiation quanta being emitted from a mycelium are strongly absorbed in situ. One can further suppose that strongly coloured species exhibit very weak BCL or do not all because the quanta emitted fail to reach the photocathode of a photomultiplier. The verification of this hypothesis requires measurements of the spectral distribution of BCL. Moreover, one can suggests experiments with melanotic i. e. a "black", melanin — abundant mutants and their amelanotic i. e. "white", pigment — deficient counterparts.

Recently, Kobayashi and Ito (1976) have illuminated *Saccharomyces cerevisiae* with 510 mm wavelength light in the presence of acridine orange as a sensitizer. They suggest the participation of the singlet excited oxygen O_2 ($^1\Delta_g$) in the photodynamic inactivation and gene conversion of yeast cells. Data of this work indicate that the illumination suppresses BCL intensity of coloured species. This effect might involve a photosensitization by naturally produced dyes.

The conclusion is come to from the Table 3, that the negative effect of illumination cannot be interpreted merely as a result of the colour changes of the mycelium or medium (excluding *Trichoderma lignorum*).

One of the essential methodical aspects of this work is the statement that the Sabouraud medium gives a clear emission. The ratio I_k/I_t is equal to $1.62 \div 1.65$, i. e. the intensity of a spontaneous, ultra-weak chemiluminescence of the medium exceeds by about 60% the intensity of the bacground. This is probably caused by a slow oxidation and condensation of $-C\begin{matrix}O\\H\end{matrix}$ group of glucose with $-NH_2$ group of proteins in

Table 3

The comparison of colour and mean relative intensity of biochemiluminenscence of some selected species of fungi

	Fungus	In third day of growing						In fifth day of growing					
		dark			illuminated			dark			illuminated		
			colour of colony			colour of colony			colour of colony			colour of colony	
		I_{BCL}	on the surface	on the reverse	I_{BCL}	on the surface	on the reverse	I_{BCL}	on the surface	on the reverse	I_{BCL}	on the surface	on the reverse
Fungi emitting BCL	*Boletus edulis*	22.12	white	cream	— 9.45	white	cream	65.03	white	cream	— 9.69	white	cream
	Microsporum gypseum	52.18	white	bright-yellow	−17.72	bright-yellow	bright-yellow	57.26	yellow-orange	yellow-orange	−13.48	yellow-orange	yellow-orange
	Pestalotia funerea	63.45	white	yellow	— 3.58	white	yellow	56.85	white	yellowish brown	−10.00	white	yellowish brown
Fungi quenching BCL	*Aspergillus nidulans*	— 9.57	bright-yellow	yellowish-green with rusty stain	−39.40	greenish-gray	greenish-brown	−10.86	yellow-green	rusty	−27.38	green	brown
	Aspergillus quadrilineatus	−17.18	white	yellow-rust	−22.66	greenish-brown	rusty	−17.38	greenish-brown	orange-brown	−19.80	greenish-brown	orange-brown
	Macrophoma candollei	— 6.26	greenish-brown	greenish-brown	−22.67	greenish-brown	greenish-brown, yellow spots	−15.56	black	dark-brown	−11.76	dark-brown	dark-brown
	Penicillium sp.	— 8.72	gray-greenish	yellow-rust	−28.94	gray-greenish	yellow-rust	−14.17	bright-turguoise	yellow	−26.76	bright-turquoise	yellow
	Tricholoma equestre	−10.41	white	cherry	−40.05	bright-gray	cherry	−18.40	yellowish-green	cherry	−37.23	yellowish-green	cherry

BCL — biochemiluminescence I_{BCL} — relative intensity of bioluminescence (average A-values calculated for 3 or 5 days of growing)

so-called "Maillard melanogenic reaction", which results in the formation of brown polymers. Gnerally, the chemiluminescence from a medium is a negative phenomenon since it masks a weak BCL of some fungi.

Negative A values may be caused by two factors, namely: 1) a strongly coloured mycelium screens (masks) the emission of the medium, in other words, the mycelium absorbs radiation quanta emitted by the medium. In such a case I_p is lower than I_k. 2) substances excreted by growing mycelium into a medium may lead to the radiationless deactivation of electronic excited states of the light emitting molecules (physical quenching) or may inhibit chemical reaction, which generate excited molecules (chemical quenching).

The above considerations lead to the methodical conclusion that in eventual experiments on weak BCL of fungi, media with possibly low I_k values ought to be used.

CONCLUSIONS

1. *Boletus edulis*, *Pestalotia funerea* and *Microsporum gypseum* clearly exhibited ultra-weak biochemiluminescence, whose intensity exceeds by 100-130% that of control, i. e. the Sabouraud medium.

2. *Aspergillus quadrilineatus*, *Macrophoma candollei*, *Penicillium sp.* and *Tricholoma equestre* did not revealed, but even diminished BCL intensity. At the time of spore production, colonies of these species became dark-coloured, or they stained the Sabouraud medium dark-brown, as it was a case in the growing mycelium of *Tricholoma equestre.*

3. The illumination of fungi cultures with 220 lx white light resulted in the decrease of BCL intensity below the level of ultraweak chemiluminescence of the control medium.

4. The Sabouraud medium used in these experiments revealed a spontaneous, ultra-weak chemiluminescence with intensity higher than that of the background. This fact limits the possibilities to measure BCL of weakly emitting fungi species.

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Biochemiluminescencja niektórych grzybów

Streszczenie

Zbadano zdolność do emitowania ultrasłabej biochemiluminescencji dwunastu gatunków grzybów rosnących na pożywce Sabouraud w zaciemnionym i oświetlonym termostacie. Stosując czułe urządzenie do zliczania fotonów, mierzono natężenie biochemiluminescencji w ciągu dziesięciu dni rozwoju kultur.

Boletus edulis, *Pestalotia funerea* i *Microsporum gypseum* wykazywały biochemiluminescencję, natomiast pozostałe gatunki, takie jak: *Aspergillus nidulans*, *A. quadrilineatus*, *Beauveria bassiana*, *Macrophoma candollei*, *Mucor lausanensis*, *Paecilomyces farinosus*, *Trichoderma lignorum* i *Tricholoma equestre*, nie dawały mierzalnej luminescencji. Oświetlenie tłumiło biochemiluminescencję, stymulując wytwarzanie barwnych związków zarówno w grzybni, jak i w pożywce.