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A new bioluminescent fungal system

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Abstract. A new bioluminescent fungal system from a wood sample with a characteristic emission around 518 ± 1 nm is described. This study indicates that water is not only important for emission but has a function in the kinetics of the reaction.

Keywords. Bioluminescence; fungal bioluminescence; *in vivo* kinetics.

Introduction

Bioluminescence is the emission of visible light by a living system. In this process, the chemical energy of an exergonic reaction is converted into light energy via a mechanism in which one of the components of the system is raised to an electronically excited state. Bioluminescent systems (Harvey, 1940, 1952) are quite diverse in nature (e.g. bacteria, fungi, algae, firefly, worms, jelly fish, cypridina, pholas etc). The process of light emission could be continuous (bacteria, fungi) or may occur in brief flashes (firefly and dinoflagellates) and it may be intracellular (bacteria, fungi, firefly) or be extra-cellular with the chemical compounds in the medium in which the reaction occurs (jelly fish).

The word luciferin and luciferase are generic names and are used for the active materials of the luminescent system even now. So far, the luciferin from a limited number of bioluminescent organisms have been isolated and characterised. The luciferin from firefly (Bitler and McElroy, 1957) and cypridina (Shimomura *et al.*, 1957) have been isolated and their structure determined. The cell-free light emission from extracts of bacteria (Hastings *et al.*, 1966) and fungi (Airth and Forester, 1962; Wassink and Kuwabara,1966; Kuwabara and Wassink, 1966) have also been studied and the mechanism of light emission proposed. The examples of light emission from the so called shining wood have been known for more than a century and is believed to be due to the fungus growing on it (Harvey, 1940). The luminous fungi (Airth and Foerstar, 1962; Wassink and Kuwabara, 1966) known so far radiate in the visible region with a maximum at $\simeq$ 528 nm.

Abbreviation used: IR, infra-red.

We report in this paper, the characteristics of light emission *in vivo* for different samples of the shining wood obtained from different trees which become luminescent during the monsoon, from a village near Lonevala in Maharashtra. Water and oxygen are essential for the emission. Further the emission intensity increases on keeping the wood sample in water for an optimum period.

Materials and methods

The various samples of the luminescent wood studied weighed between 2 to 3g. The luminescent emission from the samples was recorded with a photomultiplier tube having S-11 response (Type EMI 9514 S). A negative potential of 1000 volts was applied to the photomultiplier tube and the output was recorded on a strip chart recorder through a direct current amplifier. The spectral characteristic of the light emitted by the sample was analysed with a 0.25 meter Jarrell-Ash-Ebert monochromator (model 82-410). The sample was placed in front of the entrance slit of the monochromator and at the exit slit end, a photomultiplier tube having S-20 response (EMI 9558 QB type) was mounted. Light emission spectrum was scanned with the help of a small motor coupled with the grating of the monochromator. The grating motor and the chart movement of the recorder were controlled by a common master switch. The chart speed used was 5cm/min and the spectrum was scanned at the rate of 100 nm/min. The calibration of the recording unit was first checked with low pressure Hg discharge lamp which gives discrete characteristic mercury lines. The accuracy of the measurement is ± 1 nm.

Results

Intensity of light emission

The intensity of light emission was measured by keeping a small piece of wood dipped under water. The build up and decay curve of the light emission is shown in figure 1. The curve in figure 1 indicates that the emission builds up fast, reaches a maximum value after about 20 min and starts decaying slowly.

Effect of water content on light intensity

A piece of wood sample was soaked with water completely by keeping it under water (water was changed after every 2 days) for 15 days. The sample was taken out from the water container and the light intensity measured. The sample was weighed immediately after the light intensity measurement. The water from the sample was allowed to evaporate with time and periodically the light intensity and the sample weight were measured. A plot of sample weight (wood + water) against the light intensity is shown in figure 2. The data in this figure clearly indicates that the intensity of light decreases with the loss of water from the wood.

Emission spectrum

The emission spectrum of the light from the wood was recorded by the arrangement already described in the experimental part and is shown in figure 3 The emission spectrum in this case is centered around 518 nm.

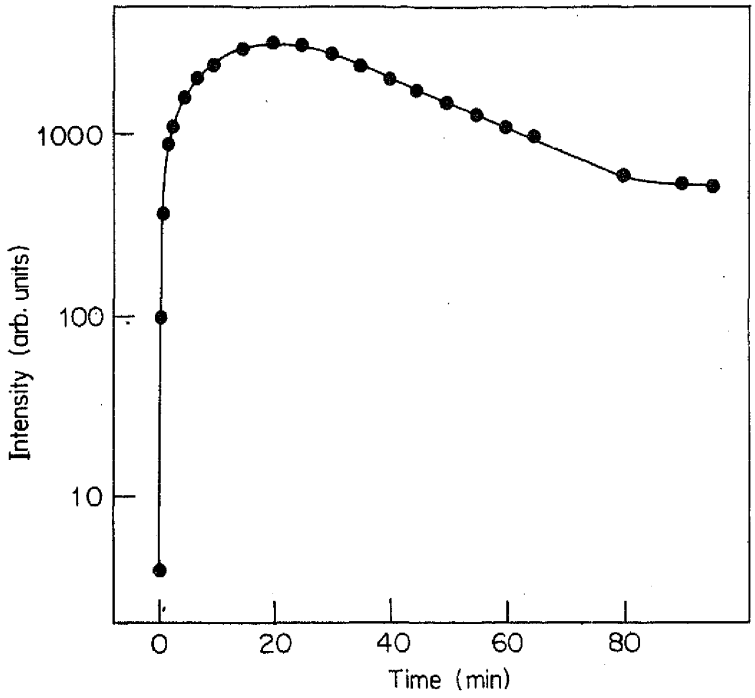

Figure 1. Plot of light intensity versus time when the sample was kept in water during measurement.

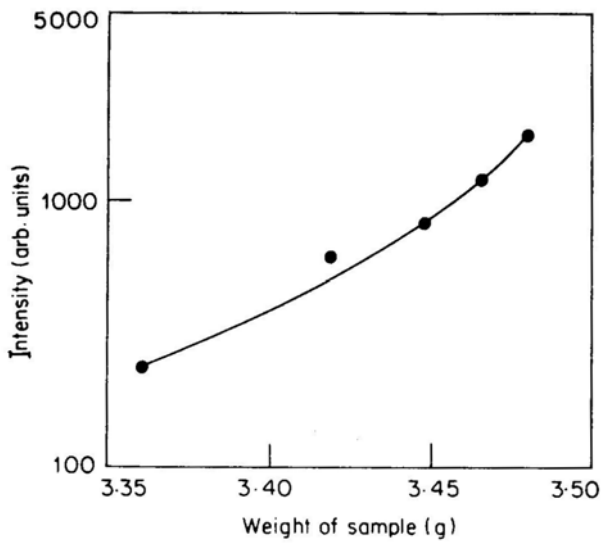

Figure 2. The dependence of light intensity on water content of the sample.

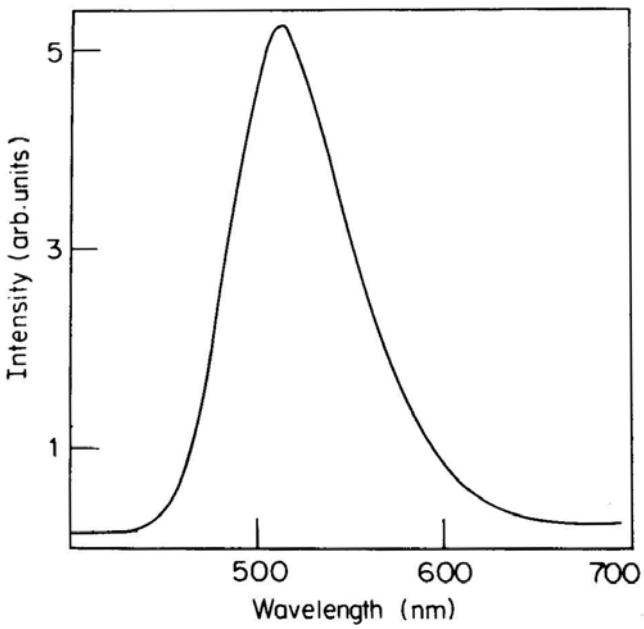

Figure 3. The emission spectrum of the luminous wood.

Effect of nitrogen gas on light emission

During the measurement of intensity of light emission in one of our experiments, nitrogen gas was passed through the chamber containing the sample. The intensity of light emission dropped slowly but could be regained once the nitrogen flow was stopped. The effect of nitrogen flow on the light intensity as shown in figure 4

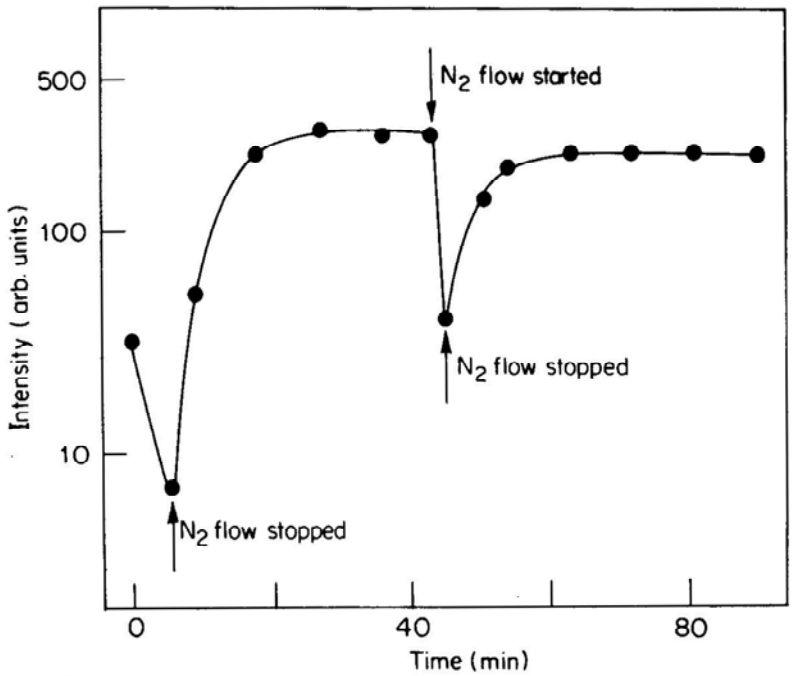

Figure 4. Effect of the presence of N_2 gas on the light intensity.

clearly indicates that light emission requires the presence of oxygen. Secondly the time required to regain the initial intensity depends on the time for which the nitrogen gas has been flowing through the chamber.

Effect of infra-red radiations on light emission

The sample of wood was taken out of water and was exposed to infra-red (IR) radiations from an infra-red lamp (250 w) for two intervals (one after another) of 10 min each. The intensity of light was measured after exposure to IR radiations by keeping the sample dipped under water. The emission intensity decreased with the increasing exposure to IR radiations as shown in figure 5. The results of the figure 5

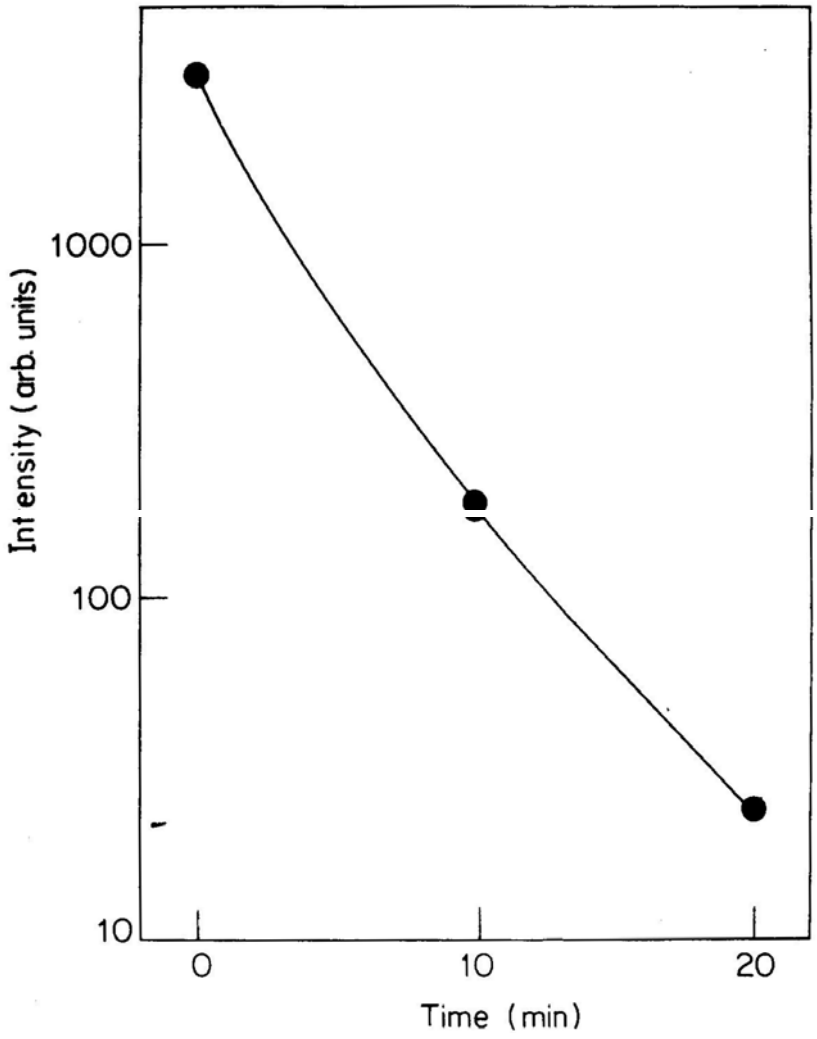

Figure 5. Effect of IR radiations on the light intensity.

suggest that the intensity of emission depends on something present on the wood which is sensitive to IR radiations.

Effect of long chain aliphatic aldehyde on light emission

The effect of long chain aliphatic aldehyde (decyl aldehyde) on the light intensity was investigated. For this purpose, during the recording of light intensity, a freshly prepared saturated solution of decyl aldehyde in water at 25°C was poured on to the sample. As a result of this, there was a continual decrease in the light intensity as shown in figure 6.

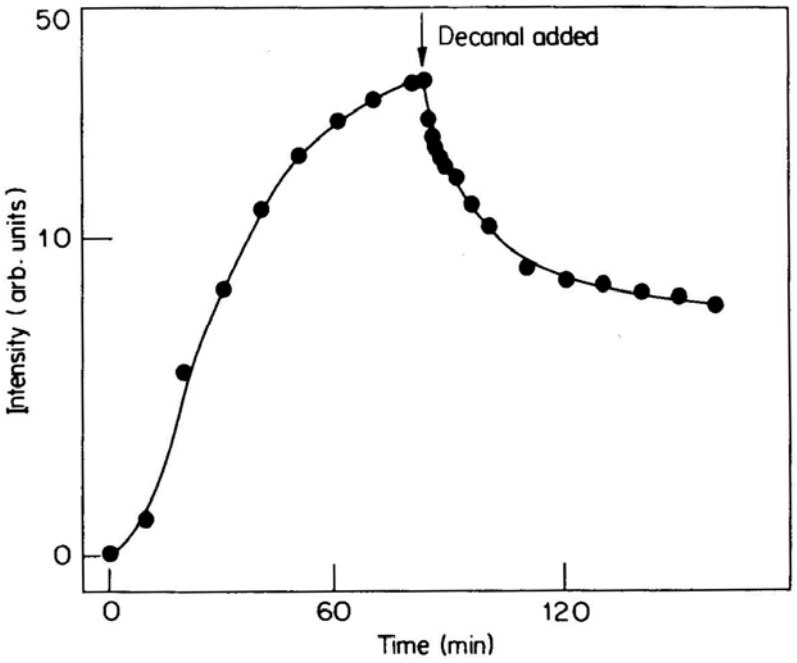

Figure 6. Effect of decyl aldehyde on emission.

Effect of antibacterial antibiotics on light emission

The effect of antibiotics chloramphenicol, actinomycin D and puromycin on the light emission was investigated with the purpose of characterising the nature of the light emitting system. The sample was kept dipped in water containing the above mentioned antibiotics (30 μM) individually and the light intensity was measured as described earlier. None of these antibiotics had any detectable effect on the light intensity over a period of 100 h.

Effect of antifungal antibiotics on light emission

The effect of fungal antibiotics griseofulvin and cycloheximide on light emission was also investigated. For this purpose, the sample was kept dipped in water containing the above mentioned antibiotics (20 μM). The light intensity was found to decrease gradually and after 100 h, decreased to almost to the background level.

In vivo kinetics

The kinetics of the light emission were followed by studying the intensity of light

emission as a function of time for the varying contents of water. The water content was varied in terms of the time for which the wood was kept dipped in water. A single sample of almost dried wood (but still weakly emitting) was kept under water for varying intervals of time. The light intensity built up and decay was measured over a constant period of 3 h. The intensity of light was measured after taking out the sample from the water. The period of measurement was not included in the total time of the different readings. The results of these experiments are presented in figure 7. One feature of the data is that the maximum in the light

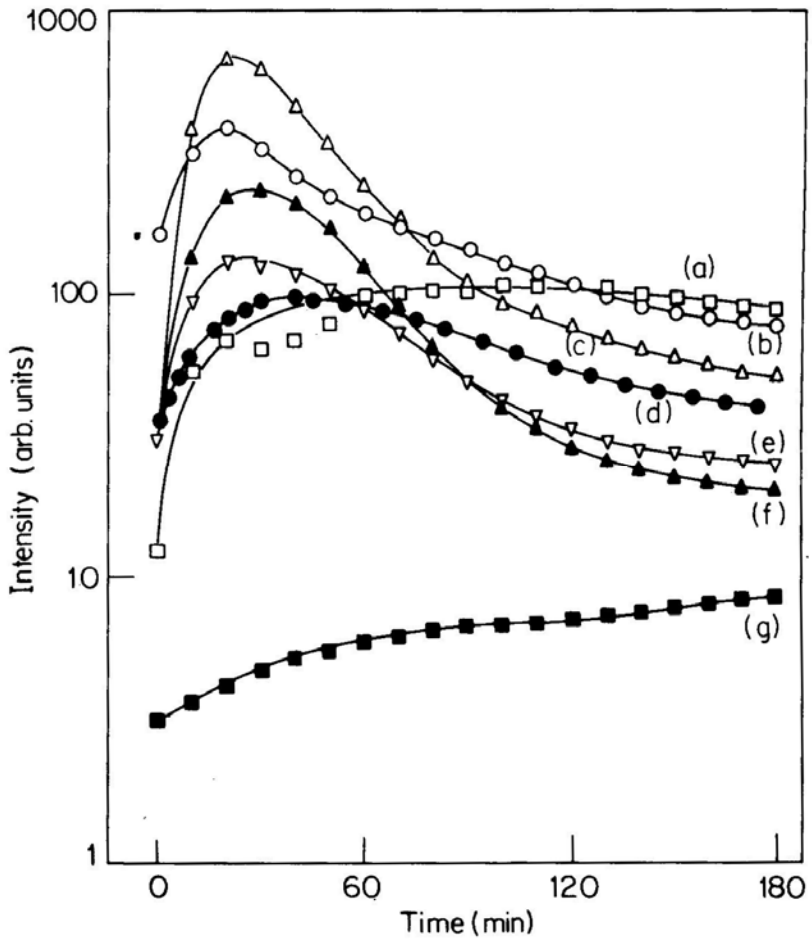

Figure 7. Plot of light intensity versus time for a sample which had been kept in water for different intervals of time before measurements.

a –❑ 346 h; b– O 165 h; c –Δ 76 h; d – ● 1 h; e–∇ 3 h; f – • 8 h ; g – ■ 10 h.

intensity reaches after the wood had been dipped in water for 76 h. Further, the light intensity attains a maximum value in around 20 min irrespective of the time the sample is kept under water. Another prominent feature of the curves in figure 7 is that the time required to increase the intensity is quite short while the decay rate is slower.

Discussion

In the present study it was observed that emission could not be revived immediately in a piece of wood which had dried but was emitting weakly. However, the emission could be revived after a few hours if the wood was kept dipped under water. This observation suggests that the cause of the shining wood could be bioluminescent in nature. The bioluminescent emission is also supported by the IR irradiation experiment, since the irradiation with IR decreases the intensity of light indicating the lethal effect of IR radiations on the living organisms. These observations are consistent with those reported by Harvey (1940) for the fungal/bacterial bioluminescent systems. Although these two bioluminescent systems have their characteristic emissions $\simeq$ 528 nm (fungal) and $\simeq$ 490 nm (bacterial), the general features of their emission are quite similar. Since the maximum in two cases occur at different wavelengths, it is suggested that different molecular species are responsible for the emission. The general nature of the emission spectrum in the present case resembles that recorded by others for luminous fungi or bacteria (Wassink and Kuwabara, 1966). However, the, λ_{max} does not correspond to either of these systems. Therefore it is apparently a new bioluminescent system with emission centered around 518 nm. The long chain aliphatic aldehydes are known to have a stimulating effect (Hastings, 1966) *in vitro* on bacterial emission. This effect has been used as one of the indices for distinguishing between the bacterial or fungal system responsible for the luminescence of the wood in the present case. The inhibitory effect of decyl aldehyde (figure 6) *in vivo* suggests that the bacterial system may not be implicated in the light emission from the sample of wood. This was supplemented from the experiment with antibacterial antibiotics which did not have any effect on the intensity of light emission. These two experiments with decyl aldehyde and antibacterial antibiotics suggest that bacteria may not be responsible for luminescence from the shining wood. All these data indicate that the bioluminescence responsible for the shining wood is probably due to a fungus that grows on moist wood. This was confirmed by the inhibitory effect of the antibiotics like griseofulvin and cycloheximide (Cochrane, 1965) which are established fungal antibiotics. This deduction is broadly in agreement with the earlier studies on shining wood (Harvey, 1940) for which fungal system has been determined to be responsible for light emission.

Till todate, the reasons for the shining of wood have been ascribed to luminous fungi predominantly of Basidiomycetes types. Among these the most widely spread is probably the well known wood destroyer *Armillaria mellea.* Three different kind of luminous fungi that have been identified are *Armillaria mellea, Mycena polygramma* and *Omphalia flavida* (Kuwabara andWassink, 1966). All of these have a characteristic emission in the yellowish green ($\lambda_{max} \simeq$ 528 nm) region.

The kinetics of the light emission *in vivo* presented in figure 7 indicates that the light intensity reaches a maximum value in about 20 min after the sample has been removed from water. The maxima in the intensity as well as the decay rate both strongly depend upon the time for which the wood was kept inside the water prior

to recording its emission. The peak intensity initially increases with the increasing duration of the sample in water (figure 7, c, d, e, f). The maximum in the intensity of light emission is obtained if the sample was kept in water for 76 h. On further increasing this time, the maximum in the intensity falls continuously (figure 7, a and b). In fact the time dependence of the light intensity for varying contents of water can be divided into two regions (i) the growth region and (ii) the decay region. The growth region indicates that when the wood is kept dipped in water, the living organisms continue to multiply and reach a maximum growth in a period of 76 h. The subsequent decrease in the emission intensity might be either due to the reduction of dissolved oxygen in water or for other unknown reasons. The light intensity is measured after keeping the sample in water in which oxygen is continuously bubbled for 100 h. It is observed that the relative decrease in the emission intensity was roughly twice as large compared to that observed for a sample which is kept in water but no oxygen is bubbled. This clearly establishes that the decrease in light intensity after 76 his not dependent on the non-availability of dissolved oxygen in water. It might not be out of place to point out the fact that bioluminescent fungal systems in nature are rare compared to nonluminous fungi. This observation is in agreement with that of Foerster *et al.* (1965) who quantitatively measured the light emission from *Collybia velutipes* mycelium of varying age.

The decay region of the kinetic curves can possibly be understood as follows. The wood being opaque, only those emitting centers which are on the surface contribute to the light intensity measurements. The water content in the wood will depend on the amount of time it is kept in water. Initially the total water content in the wood is less. During the recording of the emission, the loss of water takes place by evaporation as has been clearly demonstrated in figure 2, and the water plays an active role in emission process, therefore the light intensity is expected to fall on loss of water. As the total water content in the wood (which increases on keeping it in water for longer time) increases, the loss of water taking place due to evaporation at the surface is compensated to an extent by the diffusion of water from inside the wood to its surface, consequently the emission decays at a slower rate. When the sample is saturated with water, the evaporation and diffusion rates probably match and a very slow decay is observed as in figure 7,a. These results clearly demonstrate that the presence of water is not only essential for detecting the emission but also the kinetics of emission depends upon the total water content present in the wood.

It can be concluded from the present study on the shining wood that a new luminous fungal system with a characteristic emission maximum at 518 nm is present. In order to understand the mechanism of light emission, a detailed study involving the isolation of luciferin and luciferase is required. The culture conditions for the growth of the fungal system for this purpose is in progress.

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