BIOLUMINESCENCE IN MUSHROOM AND ITS APPLICATION POTENTIALS

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ABSTRACT
Bioluminescence is a biological process through which light is produced and emitted by a living organism resulting from a chemical reaction within the body of the organism. The mechanism behind this phenomenon is an oxygen-dependent reaction involving substrates generally termed luciferin, which is catalyzed by one or more of an assortment of unrelated enzyme called luciferases. The history of bioluminescence in fungi can be traced far back to 382 B.C. when it was first noted by Aristotle in his early writings. It is the nature of bioluminescent mushrooms to emit a greenish light at certain stages in their life cycle and this light has a maximum wavelength range of 520-530 nm. Luminescence in mushroom has been hypothesized to attract invertebrates that aids in spore dispersal and testing for pollutants (ions of mercury) in water supply. The metabolites from luminescent mushrooms are effectively bioactive in anti-moulds, anti-bacteria, anti-virus, especially in inhibiting the growth of cancer cell and very useful in areas of biology, biotechnology and medicine as luminescent markers for developing new luminescent microanalysis methods. Luminescent mushroom is a novel area of research in the world which is beneficial to mankind especially with regards to environmental pollution monitoring and biomedical applications. Bioluminescence in fungi is a beautiful phenomenon to observe which should be of interest to Scientists of all endeavors.

Key words: Bioluminescence, luciferin, luminous mushrooms, applications.

INTRODUCTION

Bioluminescence comes from the Greek word '*bios'* meaning living and the Latin word '*lumen'* meaning light. So put together, you have living lights. Bioluminescence is a biological process through which light is produced and emitted by a living organism resulting from a chemical reaction occurring within the body of an organism (Weitz, 2004). These organisms use a variety of body parts to emit light in different colours and for different purposes. These myths about the origin of fire comes from the (burning of the sea) ocean which is now known to be as a result of bioluminescent planktons (the Dinoflagellates) in the ocean (Lee, 2008). Bioluminescent organisms range from marine bacteria and other plankton to corals and fishes, worms and insects, fungi and humans. The most commonly known example of bioluminescence is the firefly, which lights its abdomen during its mating season to communicate with potential mates (Weitz, 2004).

Bioluminescence occurs in 25 different phyla many of which are totally unrelated and diverse with the phylum fungi included in the list. One of the features of biological light that distinguishes it from other forms of light is that it is cold light. Unlike the light of a candle and that of a light bulb, bioluminescent light is produced with very little heat radiation (Weitz, 2004).

HISTORY OF BIOLUMINESCENCE IN MUSHROOM

Bioluminescence in fungi (mushroom) has been known down the ages. Shimomura (2006) noted that this phenomenon was described by Aristotle as early as the 4th century B.C. and Pliny in the 1st century A.D. Aristotle called this phenomenon 'shinning wood' and 'fire fox'. Pliny the elder (23-79 AD) mentioned feasting on a glowing, sweet fungus found on trees in France and, in the late 15th century, a Dutch consul gave account of Indonesian peoples using fungi fruiting bodies to illuminate forest pathways. In Micronesia, luminous mushrooms were frequently destroyed because their presence means an ill omen (Desjardin et al., 2008; Vladimir et al., 2012).

According to Vladimir et al. (2012) many Scientists made observations and notations of luminous earth. Early observations on luminescence were attributed to interactions of insects or animal. Report on light from luminous wood due to fungi was recorded in a study of luminous timbers on luminescence in mines by Bishoff in 1823. This study paved way for further research by several scientists which established room for modern experimental work on bioluminescent fungi by 1855 to include:

1) The light without heat
2) The light ceased in vacuum in presence of hydrogen and carbon dioxide
3) The light was independent of humidity, temperature, light, and do not burn any brighter in pure oxygen (Vladimir et al., 2012).

The luminescent parts of the mushroom included the pileus (cap), hymenium (gills), and the mycelia threads in combination or separately. Similarly, the individual spores were seen to be luminescent. Shimomura (2006) noted that the aboriginals of Micronesia used luminescent mushrooms on their heads as decorations for ritual dances or crushed them to paint their faces to intimidate enemies, although, luminous mushrooms were frequently destroyed because to find them was considered an ill omen (Desjardin et al., 2008). As stated by Desjardin et al. (2008) all known bioluminescent fungi came from the basidiomycetes belonging to three distinct lineages. Twelve (12) species occur within the *Omphalatus* lineage, five (5) in *Armellaria* lineage while forty seven (47) in Mycenoid lineage. Luminous mushrooms have been found in North and South America, Europe, Asia, Australia and Africa hence the list keeps steadily increasing as more and more new species are found both in subtropical and tropical zones of the globe where natural conditions are most favourable for their habitation (Vladimir et al., 2012).

MECHANISM OF MUSHROOM BIOLUMINESCENCE

Like other organisms in which it occurs, bioluminescence in mushroom is an oxygen-dependent reaction involving substrates generically termed luciferins, which is catalyzed by one or more of an assortment of unrelated enzymes referred to as luciferases (Perry, 2007). During luciferic-luciferase reaction, unstable chemical intermediates are produced. As these intermediates decompose, excess energy is released as light emission, causing the tissues in which this reaction occurs to glow or luminesce (Perry, 2007).

The reaction involves the following elements;
1) Enzymes (luciferase) – a biological catalyst that accelerates and control the rate of chemical reaction in cells.
2) Photons – packs of light energy.
3) ATP – Adenosine triphosphate, the energy storing molecule of all living organisms.
4) Substrates (luciferin) – a specific molecule that undergoes a chemical change when affixed by an enzyme.
5) Oxygen – as a catalyst.

A simplified formula for the bioluminescent reaction is;

ATP (energy) + luciferin (substrate) + luciferase (enzyme) + O_2(oxidizer) = light (photon).

The bioluminescent reaction occurs in two stages;
1. The reaction involves a substrate (D – luciferin), combining with ATP and oxygen, which is controlled by the enzyme (luciferase).
2. The chemical energy in stage 1 excites a specific molecule (the luminescent molecules; the combining of luciferase and luciferin). The excitement is caused by the increased energy level of that of the luminescent molecule. The result of this excitement is decay which is manifested in the form of photon emissions that produces the light. The light given off does not depend on external light or other energy taken in by the organism and is just the by-product of the chemical reaction and is therefore cold light.

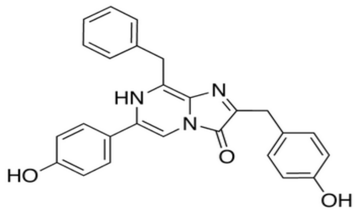
The energy in photons can vary with the frequency (colour) of the light. Different types of substrates (luciferins) in organisms produce different colours. Hence, the various luminous organisms have their own different colours. Fungi emit greenish bluish light, marine organisms emit blue light, and fire flies emit greenish yellow and so on.

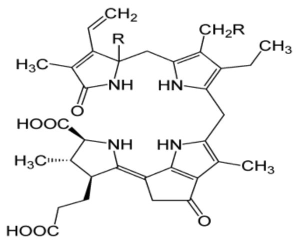
THE LUMINESCENT MOLECULE

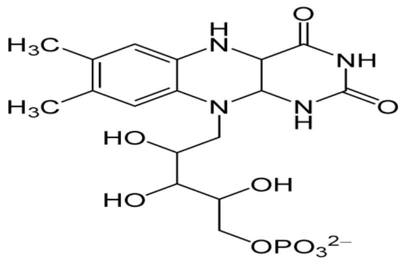
Luciferin is a chemical substance found in the cells of bioluminescent organisms. It is gotten from the Latin word '*lucifer'* meaning light – bringers. It is a generic term for the light emitting compound found in organisms that generate bioluminescence. It is a natural substrate for the enzyme luciferase that catalyzes the production of the typical green light of mushrooms and firefly (Meroni et al., 2009). When luciferin is oxidized under the catalytic influence of luciferase and ATP, a bluish green light is produced. Because the reaction is dependent on ATP, it allows researchers to determine the presence of energy of life (Meroni et al., 2009).

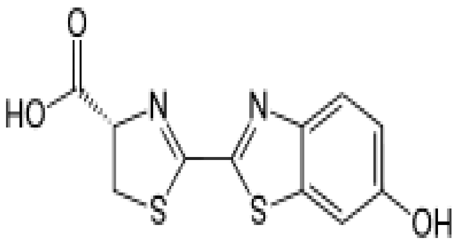
The isolation of luciferin from luminescent mushrooms has not yet been accomplished (Sun, 2009). Only three luciferins structures have been elucidated; namely firefly luciferin, cypridina luciferin and latia luciferin. No relationship is apparent from the structures of these luciferins (Goro, 2010). Although luciferin-luciferase bioluminescence is found in hundreds of taxa across many phyla, there are five basic luciferin-luciferase systems; bacterial luciferin, dinoflagellate luciferin, vargula luciferin, coelenterazine and fire fly luciferin which have been found to be similar to the luciferin isolated from *Mycena citricolor* cultivated mycelium (Desjardin et al., 2008) (Figure 1).

Luciferin isolated from organisms are


Coelenterazine


Dinoflagellate luciferin


Bacterial luciferin


Firefly luciferin

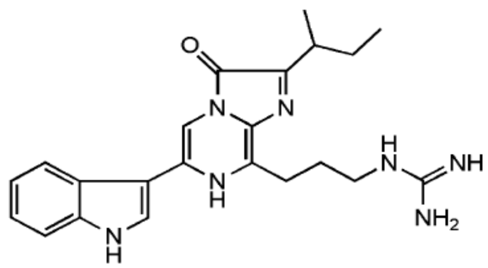

Vargula luciferin

Figure 1. Luciferin isolated from various luminescent organisms.
Source: Desjardin et al. (2008).

applicable in various forms; including their applications in variety of *in vitro* assays to monitored the production of light using luminescent or scintillation counter. It is used in the monitoring of the production of light *in vivo* (Meroni et al., 2009). Luciferase is applicable in the conversion of D-luciferin to produce bioluminescence applicable in real time - low imaging of gene expression in cell cultures, individual cells, whole and transgenic organisms (Meroni et al., 2009). Luciferin obtained from fire fly is particularly a good reporter for *in vivo* biophotonic imaging due to properties of its emission spectrum (Meroni et al., 2009).

NATURE OF BIOLUMINESCENT MUSHROOM

More than two-thirds of these bioluminescent species are members of the diverse and widespread genus *Mycena*. Other genera containing luminescent species include *Armillaria, Omphalotus* (including *Lampteromyces* and luminescent *Pleurotus* species), *Gerronema, Panellus* and *Dictyopanus* (Table 1). Luminescence may occur in both mycelia and fruiting bodies, as in *Panellus stipticus* and *Omphalotus olearius* or only in the mycelia and young rhizomorphs as in *Armillaria mellea* (Wietz, 2004). In *Mycena lamprospora* however, it is the mature spores that has been observed to luminesce. In many instances, it is the hyphae present in decaying plant tissues that luminesce, resulting in the appearance of luminescent wood or leaves (Wietz, 2004).

It is common knowledge that bioluminescent

Table 1. Various examples of luminescent mushroom.

Binomial Name	Common name	Luminescent part	Distribution
Armillaria fuscipes Petch	Nil	Mycelium	Malaysia
Armillaria gallica Marxm.& Romagn.	Nil	Mycelium	Europe, North America
Armillaria mellea (Valh.) P.Kumm.	Honey fungus	Mycelium	Europe, North America
Armillaria solidipes (Romagn.) Henrik	Nil	Mycelium	Europe, North America
Armillaria tabescens (Scop.) Emel	Nil	Mycelium	Europe, North America
Gerronema viridilucens Desjardin, Capelari & Stevani	Nil	Mycelium and fruiting bodies	South America
Mycena asterina Desjardin, Capelari & Stevani	Nil	Fruiting bodies	South America
Mycena citricolor (Berk. & M.A. Curtis) Sacc.	Nil	Mycelium	South America, Central America
Mycena chlorophos (Berk. & M.A. Curtis) Sacc.	Puff balls	Mycelium and fruiting bodies	Malaysia, Japan, Pacific Islands
Mycena daisyogunensis Kobayasi	Nil	Fruiting bodies	Japan
Mycena discobasis Metrod	Nil	Fruiting bodies	South America, Africa
Mycena epipterygia (Scop. Fr.) S.F.Gray	Nil	Mycelium	Europe, North America, Japan
Mycena fera Maas Geest. & de Meijer	Nil	Fruiting bodies	South America
Mycena galopus (Pers.: Fr.) P.Kumm.	Milk drop mycena	Mycelium	Europe, North America, Japan
Mycena haematopus (Pers.: Fr.) P.Kumm.	Bleeding fairy helmet	Mycelium and fruiting bodies	Europe, North America, Japan
Mycena illuminans Henn.	Milking mycena	Fruiting bodies	Malaysia, Japan
Mycena inclinata (Fr.) Quél.	Clustered bonnet	Mycelium	Europe, North America, Africa
Mycena kentingensis	Oak stump bonnet	Fruiting bodies	Taiwan
Mycena lacrimans Singer	Nil	Fruiting bodies	South America
Mycena lamprospora (Corner) E.Horak	Nil	Spores	Malaysia, Australia
Mycena lucentipes Desjardin, Capelari & Stevani	Glowing stem mushroom	All parts	South America, Central America and the Caribbean
Mycena luxaeterna B.A.Perry& Desjardin	Eternal light mushroom	Mycelium and fruiting bodies	South America
Mycena maculata P.Karst.	Reddish spotted mycena	Mycelium	Europe, North America, Africa
Mycena manipularis (Berk.) Métrod	Nil	Mycelium and fruiting bodies	Australia, Malaysia, Pacific islands
Mycena noctilucens Kawam. ex Corner	Nil	Fruiting bodies	Malaysia, Pacific islands
Mycena olivaceomarginata (Masseeapud Cooke) Massee	Nil	Mycelium	Europe, North America
Mycena polygramma (Bull.: Fr.) S.F.Gray	Grooved bonnet	Mycelium	Africa, Europe, North America, Japan
Mycena pseudostylobates Kobayasi	Nil	Mycelium	Japan
Mycena pura (Pers.: Fr.) P.Kumm.	Nil	Mycelium	Europe, North America, South America, Japan
Mycena rosea (Bull.) Gramberg	Rosy bonnet	Mycelium	Europe
Mycena sanguinolenta (Alb.&Schwein.: Fr.) P.Kumm.	Bleeding bonnet	Mycelium	Europe, North America, Japan
Mycena stylobates (Pers.: Fr.) P.Kumm.	Nil	Mycelium	Africa, Europe, North America, Japan
Mycena sublucens Corner	Bulbous bonnet	Basidiomes	Malaysia
Neonothopanus gardneri (Berk. ex Gardner) Capelari, Desjardin, Perry, Asai&Stevani	Nil	Mycelium and fruiting bodies	South America
Neonothopanus nambi (Speg.) Petersen &Krisai-Greilhuber	Nil	Fruiting bodies	Australia, South America, Central America and the Caribbean, Malaysia
Nothopanus noctilucens (Lév.) Singer	Nil	Fruiting bodies	Japan
Omphalotus illudens (Schwein.) Bresinsky&Besl.	Jack-o-lantern mushroom	All parts	Europe, North America
Omphalotus japonicus (Kawam.) Kirchm. &O.K.Mill.	Moon light mushroom	All parts	Japan
Omphalotus mangensis (J.Li& X.Hu) Kirchm. &O.K.Mill.	Nil	Fruiting bodies	China

Omphalotus nidiformis (Berk.) O.K.Mill.	Ghost fungus	Gills	Australia
Omphalotus olearius (DC.: Fr.) Singer	Jack-o-lantern mushroom	Gills	Europe
Omphalotus olivascens H.E.Bigelow, O.K.Mill.&Thiers	West jack-o-lantern	Fruiting bodies	North America
Panellus luminescens (Corner) Corner	Nil	Mycelium and fruiting bodies	Malaysia
Roridomyces roridus	Dripping bonnet	Spores	North America, South America
Panellus stipticus (Bull.: Fr.) P.Karst.	Bitter oyster	Gills	Australia, Africa, Europe, North America, South America, Japan

Source: Desjardin et al. (2008).

mushrooms are generally saprophytes (less frequently-pathogen) of plants all of which are white-spored Basidiomycetes. At different stages of their life cycle they emit greenish light with maximum wavelength range of 520-530 nm (Shimomura, 2006; Desjardin et al., 2008; Bondar et al., 2011). A luminous mushroom emits light only for a certain period (periods) of its life cycle; after and before that period, it practically does not glow (Shimomura, 2006). Dao (2006) observed that light emittion from the mycelia of *Omphalotus af. illudens* in open air (oxygen) was maximum after 5 h, fall down to 13th hour and eventually stop emitting light (Figure 2). On the whole, the luminescence of younger fruiting bodies and young actively growing mycelium is brighter than mature fruiting bodies and old mycelium, even though the intensity of their luminescence varies widely with species and environment (Vladimir et al., 2012). The light emitted is often quite faint and typically requires very dark conditions to see. While the light or luminescence is difficult to observe in some species of *Mycena*, others are relatively brighter, bright enough to read and can be visible from a distance of up to 40 m (Perry, 2007).

Panellus stipticus is unusual in that luminescence is exhibited only in the North America strains of *P. stipiticus* and not by the Eurasian strains (Weitz, 2004). Vladimir et al. (2012) noted that wood destroying fungi are specified by substantially higher luminescence (visible luminescence) and intensity of these species correlates with the activity of enzymes of the secondary metabolism participating in lignin destruction. They found no correlation between the edibility of the mushroom and its bioluminescence.

APPLICATION POTENTIALS OF BIOLUMINESCENCE IN MUSHROOM

Generally, functions of bioluminescence in organisms have been to attract prey, to detect prey,

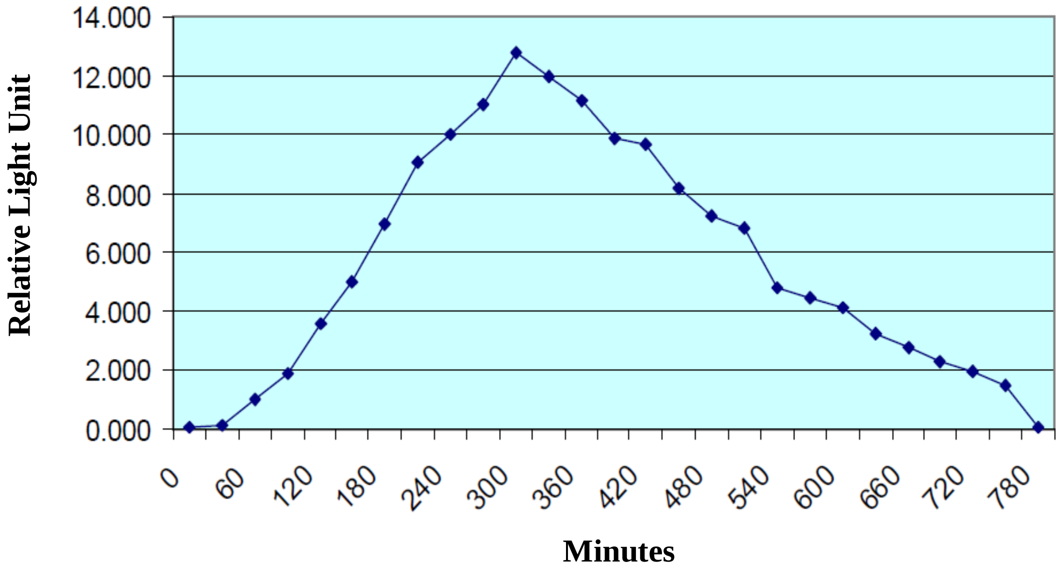


Figure 2. Light intensity from mycelia of *Omphalotus af. illudens* in open air following time.
Source: Dao (2006).

to defend oneself against a predator, to communicate between same species and to navigate. In fungi, luminescence has been hypothesized to attract invertebrates that aid in spore dispersal (Desjardin, 2008). This is especially adaptive in closed canopy forests where wind dispersal is hindered. In application of the saying…*befriend the enemy of your enemy*, some mushrooms glow to attract the predators of fungivores, repulsion of phototropic fungivores, and as a warning signal to nocturnal fungivores (Desjardin et al., 2008; Weitz, 2004). This application is especially useful to animal and environmental biologists who study these fungivores (invertebrates), hence mushrooms can be used as a lure for trapping these insects.

During the Vietnam War, to avoid being spotted by enemy aircraft, the Vietnamese army used to wear luminescent chunks of rotting wood behind their hat to keep track of each other while operating at night in the forest. In these chunks, mycelia system of luminescent mushrooms grows strongly. Local people have collected and put luminescent fruit-bodies in plastic bags to use them for illumination at night (Dao, 2006). The feasibility of employing bioluminescent mushrooms as an indicator test object for bioassays has been studied. *Armillaria mellea, Mycena citricolor, and Gerronema viridilucens* were used to develop toxicity test (Weitz, 2004;Horsewell et al., 2005; Mendes, and Stevani, 2010).

Many scientific researchers have developed luminescent mushrooms into applications in testing for pollutants [ions of mercury (Hg)] in water supply when concentrations are too low to detect by conventional means (Dao, 2006; Wan et al., 2014). Luminous mushrooms were used to detect toxicity of 3,5-dichlorophenol, pentachlorophenol and salts of heavy metals. These compounds have been shown to inhibit the luminescence of mycelium of *Armillaria mellea* and *Mycena citricolor*. The luminous mushrooms are of real interest as luminescent markers in developing new luminescent microanalysis methods for biology, biotechnology and medicine. The light generated by this reaction has been utilized by scientists as a bio indicator or biosensor for tuberculosis as well as heavy metals (Vladimir et al., 2012). As it is an oxygen-dependent reaction, it has been hypothesized that bioluminescence may have evolved as a method to consume excess oxygen produced in the cells of organisms during other metabolic processes (that is an antioxidant). The luminescent molecule can be used in lux-gene transformation into living organism to make desired luminescent organism (Dao, 2006).

The metabolite from the luminescent mushrooms present the effectively bioactive terpenoids such as Nambinones A-C, 1-epi-nambionone B and Nambinone D compounds which possess anti-mould, anti-bacteria, ant-virus and especially in inhibiting growth of cancerous cells. Therefore, a number of metabolites were isolated from fungi which found their way into medical applications as natural products, starting material for pharmaceuticals or as lead structures for the development of pharmaceutical products (Dao, 2006; Mehmet and Gulsen, 2015).

CONCLUSION AND RECOMMENDATION

We live in the era of illumination. Luminescent mushroom is a novel area of research in the world which would be beneficial to mankind especially with regards to environmental pollution, gene-transformation, pharmaceutical, biochemical and biomedical application. Bioluminescence in fungi is a beautiful phenomenon to observe which should be of interest to Scientists of all endeavors.

Mushrooms occur abundantly in our forests and terrestrial environment. Luminescent mushrooms should be identified and cultivated by mycologists. Collaborative work should be encouraged among biochemists, chemists and physicists on the isolation and purification of Luciferin compounds present in these mushrooms and other possible potentials in production of light. Due to unavailability of light supply in Nigeria, luminescent mushrooms may be an alternative source of illumination in our local communities at night.

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