Biological, chemical and ecological properties of *Armillaria mellea* (Vahl) P. Kumm

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Streszczenie:

Armillaria mellea is a commonly occurring fungi species, belonging to the *Basidiomycota* division. As an edible fungus, *A. mellea's* fruiting bodies and rhizomorphs are rich in high amounts of polysaccharides, proteins, fats and other elements. The species has also been found to be a medicinal fungus as its curing properties have been proven by results of many pieces of research. *A. mellea* shows a number of interesting abilities, mainly it is capable of bioluminescence and forms mycelial cords. These are a tool for conducting nutrients but also infecting adjacent plants, which makes the species a dangerous plant pathogen. Due to the ability of degrading wood components with the use of the enzymatic ligninolytic complex, the fungus has been classified as a member of a wide fungal group called *white-rot fungi*. Fungi belonging to this group are capable of breaking down many hazardous environment pollutants, which raises an argument to apply them in mycoremediation.

Słowa kluczowe: *Armillaria mellea*; Rhizomorphs; Bioluminescence; Medicinal fungi; Plant pathogen; White-rot fungi; Mycoremediation

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Introduction

The name *Armillaria* descends from the Latin word *armillaria* which means a bracelet, a bangle or an epaulette and which relates to a characteristic of most of *Armillaria* species, a broad, persistent, skin-like ring attached to the upper part of the stipe (Gębska, 2009).

Armillaria mellea, commonly known as a honey mushroom, belongs to a wide taxonomic group with the following classification: *Fungi*, *Dikarya*, *Basidiomycota*, *Agaricomycotina*, *Agaricomycetes*, *Agaricomycetidae*, *Agaricales*, *Tricholomataceae*, *Armillaria* (Kabulska, 2015).

Mellea, though it means honey, specifically refers to the appearance of caps, not to the flavour or scent that sometimes can be misled.

It is significant to point out that though the name *Armillaria mellea* is well established in literature, the forms of the honey fungus are variable and the taxonomists are not unanimous about the interpretation of the species, hence it is common to come across in literature the phrase *Armillaria* complex (Cha et al., 1994) (Bérubé and Dessureault, 1989). The *Armillaria* complex consists of several species that are very difficult to distinguish even by specialists.

The species (young fruiting bodies) is considered to be edible when thoroughly cooked and there are a few ways of culinary processing it. Though the cases of being allergic to the honey mushroom were reported and some people might have difficulties with digesting it, hence major care should be taken when preparing and consuming it (Gumińska and Wojewoda, 1988). Literature documents the fungus to taste mild to bitter with a rather sweet scent (Lisiewska and Szmid, 1972). Fruiting bodies of fungi owe their culinary popularity to their flavour and texture as well, but mostly to their nutritional values as they are known to be healthy, low in calories, high in proteins, vitamins, some micro- and macroelements and they contain chitin, fibres and minerals (Ouzouni et al., 2009).

A. mellea is a saprophytic, parasitic and mycorrhizal fungus that belongs to a wide group called white-rot fungi. Fungi that are members of this group have the ability to degrade cellulose and lignin in any wooden material. As a saprophytic fungus its fruiting body, also known as a stump, develops on hardwood although can grow on any wooden material. The second form is a parasitical form that infects roots of living trees, causes mortality, wood decay and growth reduction. The last form is a mycorrhizal co-living with several species, e.g. *Gastrodia elata* (Kikuchi et al., 2008) (Redfern and Filip, 1991).

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Species description

Morphology and characteristics

Among the fungi within *Armillaria* genus, *Armillaria mellea* belongs to the group of fungi whose fruiting bodies show regular macroscopic characteristics. The mentioned features involve the presence of gills on hymenium that can be either adnate or subdecurrent and a ring on a stipe. A cap can be convex or flat (O'Reilly, 2011). The pileus is usually honey coloured but can differ from almost white to dark reddish brown and the surface is smooth, with no squamule. The detailed characteristics of the species are listed in table 1.

Pileus (cap)	1.6-7.5 cm in diameter, abruptly conic to convex when young, plano-convex, finally plane; surface dry, yellow to olive brown, dark yellowish brown to dark olive brown at center, sometimes very dark grayish brown, pigments scattered on the surface, somewhat covered with dark yellowish--brown fine fibers toward the center. Margin usually inrolled when young then acute later, concolorous with a cap or somewhat darker later. Flesh firm, thin to thick at center; cortex white.
Lamellae (gills)	0.6-3.8 × 0.2-0.8 cm, white to yellow toward the margin when young then yellow to yellowish brown in age, close, decurrent to falcate-decurrent.
Stipe (stalk; stem)	Central, 3.6-10 × 0.4-1.0 cm, cylindric, equal to slightly tapered from base to apex, pale yellow to yellow at the apex and very dark grayish brown to very dark brown toward the base, longitudinally fibrillose-striate, covered with fibrous scales of yellow at the apex and pale yellow toward the base, solid. Annulus membranous, thick, usually unbroken and attached to the stipe, white with yellow fibers on the back-side. A broad persistent skin-like ring attached to the upper part of the stipe.
Spores	White in mass, subglobose, broadly elliptic, with an apiculus, 10-13.5 × 6-8 µm, smooth, hyaline, nonamyloid.

Table 1. Characteristics of *A. mellea* species (Majdańska, 2007) (Cha and Igarashi, 1995a) (Lisiewska and Szmid, 1972)

What is more, *A. mellea* has the ability to produce tuber-like masses called sclerotia that are capable of sending out rhizomorphs (mycelial cords), linear aggregations of parallel-oriented hyphae resembling of cylindrical shape strands (Marshall, 2003). These branches-like flaps are 1-3 mm in diameter with a reddish brown to black outer cortex layer mostly in the upper 30 cm soil layer (Lamour et al., 2007). The mature cords are composed of wide, empty vessel hyphae surrounded by narrower sheathing hyphae. Cords may look similar to plant roots, and also often have similar functions- are capable of conducting nutrients over long distances though this is not the only function they have. Parasitic fungi, such as *A. mellea*, use rhizomorphs to invade the soil and attack the roots of the trees, this being the reason of forest's destruction as the direct contact between a diseased and a healthy tree it not needed to spread the infection within mycelial cords.

Another interesting property of *A. mellea* is bioluminescence. The light produced by *Armillaria* has been recognized and therefore documented for more than a century (Murrill, 1915). Although the mycelia and rhizomorphs of *Armillaria* are bioluminescent, light has not been observed in fruiting bodies (Mihail and Bruhn, 2007) (Mihail, 2015). The luminescence of the fungal organs is a physiological phenomenon dependent on the intracellular processes as well as the environmental conditions such as the oxygen and humidity levels (Stasiak, 2008). Fungal bioluminescence has been linked to metabolic activity due to suggestions for it to be a NAD(P)H-dependent system in *A. mellea* (Weitz et al., 2002). Some researchers believed that bioluminescence is performed to attract the spore-dispersing invertebrates or predators of fungivores (Desjardin et al., 2008) (Weitz, 2004), however, this was only a hypothesis and it seems more now that this process has no ecological value and it is just a by-product of lignin degradation (Bermudes et al., 1992).

Habitat

The literature shows that most of the *Armillaria* genus species tend to grow in the forestry regions that contain great content of the organic matter and are sufficient in humidity. *A. mellea* is a thermophilic organism, it was documented that its north border of range is most likely the Dutch coast and south border is Sicily (Majdańska, 2007) *A. mellea* is distributed on all continents but most widely in temperate regions: Britain, Ireland, North America and throughout mainland Europe, though rarely found in Scandinavia.

There are over 40 species in the genus *Armillaria* described worldwide and 5 of them can be found in Poland such as *Armillaria borealis*, *A. gallica*, *A. cepistipes*, *A. ostoyae* and *A. mellea* (Stasiak, 2008). It was reported that in these regions the highest growth of fruiting bodies is observed between July and November.

Life cycle

Among the *Basidiomycetes* it is common for the vegetative stage to be dikaryotic, nevertheless, *Amillaria* genus tend to have it diploid (Korhonen and Hintikka, 1974).

The life cycle of Armillaria begins with the fusion of two hyphae of different mating types ("+" and "-") of the haploid primary mycelium. During the interaction in the plasmogamy process, the hyphal cells become dikaryotic and they give a start for the growth of the dikaryotic secondary mycelium, which with time forms a fully grown dikaryotic fruiting body. Gills of the fruiting body's cap are lined with dikaryotic basidia, forming basidiocarps. There, in basidiocarps, the next stage, karyogamy, takes place. The fusion of nuclei occurring in basidia of a basidiocarp results in each of them having a single haploid nucleus. When this stage is finished, the basidia undergo meiosis forming four haploid nuclei which soon after give a start to four haploid basidiospores. After basidiospores being released and after meeting suitable conditions, they are ready to germinate and form again the haploid primary mycelium (Nowak, 2005).

Most *Armillaria* species are heterothallic though the results of much research show populations of *Armil-*

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laria (including *A. mellea*) that are homothallic or secondary homothallic (a type of homoheteromixis) (Abomo-Ndongo et al., 1997). The difference between heterothallism and homothallism is that in homothallic strains the four haploid nuclei fuse into two pairs of diploid nuclei which migrate to two of four basidiospores whereas, in heterothallic strains, the four haploid nuclei do not fuse, each of them locates to four uninucleate basidiospores (Wilson et al., 2015).

Components and their medicinal use

Fungi have a long well-documented history of being used for food and some traditional medicine purposes due to their components of high nutrition values and non-poisonous nature (Chang and Wasser, 2012). They found their use in herbal medicine to cure dizziness, headache, insomnia, limbs numbness and infantile convulsions and many other symptoms (Chi et al., 2013) (Zavastin et al., 2015) and they are regarded to have prophylactic properties in addition to coronary heart disease and hypertension (Bobek et al., 1995).

The discovery of penicillin has widened horizons of many researches and combined such scientific fields as mycology, chemistry, pharmacy and medicine showing that fungi have a lot to offer to the world of science (Sułkowska-Ziaja et al., 2005). Although only recently it has become a common knowledge that mushrooms are also a good material for producing any dietary supplements as well as they play an increasing role in the prevention of many dysfunctions and diseases (Phan et al., 2014).

Carbohydrates

The research data shows that *Armillaria mellea's* fruiting bodies and rhizomorphs are rich in the amount of carbohydrates (16,44 g per 100 g of a dry matter) (Stasiak, 2008). Among this wide group polysaccharides seem to be the best-known fungal metabolites due to their pharmacologic properties. Early studies on the polysaccharides obtained from *A. mellea* fruiting bodies revealed the antitumor activity of the fraction containing β-glucan with a peptide component (Amar et al., 1976) (Vaz et al., 2001). β-glucans (along with phenols and flavonoids) are also considered to have an antioxidant potential due to inhibitory activity against DPPH free radicals of its methanol and water extracts (Strapáč et al., 2016).

Protein type	Albumins	Globulins	Prolamines	Gluteins
Content (g / 100 g of a dry matter)	55.62	26.80	11.82	3.75

Table 2. The content of total nitrogen in proteins fractions (g/100 g of a dry matter) (Stasiak, 2008)

Further components of polysaccharides present in *A. mellea* fruiting bodies such as glycogen, cellulose, mannans and chitin were studied and the research results showed these compounds to reduce the risk of cardiovascular disease by lowering LDL cholesterol and triglyceride concentration (Muszynska et al., 2011). Chitins and glucans are documented to be capable of lowering blood pressure, influencing the immune system and performing hypoglycemic, antibacterial, antiviral and anti-inflammatory actions (Muszynska et al., 2011) (Pochanavanich and Suntornsuk, 2002). As well it has been reported for the polysaccharides isolated from *A. mellea* to have a protective effect on bone marrow cell damage caused by the immunosuppressant cyclophosphamide (Kim et al., 2008).

The remaining components of *A. mellea* within carbohydrates group that were not described to have any medicinal properties are trehalose, mannitol, xylose and D-erythritol (Kalač, 2009).

Proteins

Protein is another constituent of *Armillaria mellea* reported to be found in high concentrations in its fruiting bodies (23.0 g per 100 g of a dry matter) (Stasiak, 2008). Protein compounds contain more than a half of total nitrogen and their content varies in higher fungi between 19.0 and 39.0 g per 100 g of a dry matter (Źródłowski, 1995) (Florczak and Lasota, 1995).

The data shows (Table 2) that *A. mellea*'s fruiting bodies contain mostly water-soluble proteins- albumins (50%). Globulins constitute about 25% of total nitrogen, prolamines and gluteins fractions respectively about 10% and 5% of total nitrogen (Karkocha, 1964).

Sesquiterpene aryl esters

Sesquiterpene aryl esters (sesquiterpenoids) are described in the literature as the major constituents of *Armillaria mellea* fruiting bodies and mycelium. Studies conducted on this group proved most of these compounds to have antibiotic and antifungal activity (Gao et al., 2009). The strength of antimicrobial activity depends on the amount of carbon atoms within the particle and the type of the substituent (Donnelly et al., 1985). The first isolated sesquiterpene aryl ester from *A. mellea* that showed antibiotic activity was melleolide (Midland et al., 1982). Later on armillarin and armillaridin were obtained and since then more than 50 different sesquiterpenoids were isolated including armillaricin, armillaribin, armillarigin and judeol (Yang et al., 1984) (Yang et al., 1989) (Yang et al., 1990).

Sterols and Sphingolipids

Although total lipid content in *Armillaria mellea* is rather low (1.8% in comparison to 5-8% average

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in mushrooms) it is still documented to have use in medicine. Ergosterol (provitamin D) being the most common of sterols present in higher *Basidiomycetes* occurs as well in *A. mellea* along with ergosterol peroxide showing antitumor activity (Florczak et al., 2004) (Muszynska et al., 2011).

Recent studies reported the discovery of a compound isolated from *A. mellea* and within the group of sphingolipids, armillaramide. (Muszynska et al., 2011) Though sphingolipids are not a well-studied group of compounds yet they became the subject of interest for many researchers due to their promising biological properties. The results of research already conducted on some of the sphingolipids show their antihepatotoxic, antitumor and immunostimulatory activities (Gao et al., 2001).

Fatty Acids

As described before, the content of fats in mushrooms is generally low, however, the unsaturated fatty acids constitute over 70% of the total content of fatty acids (Bernaś et al., 2006). Studies conducted on *A. mellea* identified 17 fatty acids present in its fruiting bodies out of which 4 were unsaturated C18 compounds and the rest saturated fatty acids (Muszynska et al., 2011) (Cox et al., 2006).

Unsaturated fatty acids remain, precursors of bile constituents, are indispensable for the synthesis of prostaglandin and prostacyclin and show antiatherogenic properties, therefore, are crucial for the functioning of humans (Muszynska et al., 2011) (Bernaś et al., 2006).

Indole Compounds

The studies focused on A*rmillaria mellea*'s indole compounds content shows that the fruiting bodies of the species contain L-tryptophan (4.47 mg/100 g d.w.) tryptamine (2.73 mg/100 g d.w.) and serotonine (2.21 mg/100 g d.w.) (Muszyńska et al., 2001) (Zavastin et al.). These compounds are common to be found in extracts of fruiting bodies or mycelium of higher *Basidiomycetes* (Muszyńska et al., 2011) (Muszyńska et al., 2009).

Tryptamine acts as a non-selective serotonin receptor agonist and serotonin-norepinephrine-dopamine releasing agent (SNDRA), by interacting with MAO inhibitors can lead to death due to poisoning (Isbister et al., 2004).

Some metabolites of tryptophan were reported to injure the nervous system by having a role in the development of degenerative diseases, therefore, the doses of this drug should be monitored when administered (Stone et al., 2003).

In the brain, serotonin plays a significant role in regulating several important functions including sleep, appetite, body temperature, cell regeneration and body weight. Abnormal serotonin levels can cause problems such as suicidal tendency, obsessive compulsive disorder, alcoholism and anxiety, hence depression is treated with medications influencing serotonin reuptake in synaptic clefts (Muszynska et al., 2011) (Chattopadhyay et al., 1996).

Enzymes

Mycelia of fungi contain a high amount of enzymes due to their method of osmotic nutrition, most of the higher *Basidiomycetes* synthesise hemicellulases, cellulases and ligninases that take part in cell walls decomposition of plant tissues (Muszynska et al., 2011).

It is documented that the fruiting body of *A. mellea* is capable of producing a lysine-specific proteinase which is a member of a novel class of metalloendopeptidases (Gao et al., 2009). This enzyme is a subject of interest because of its potential fibrinolytic activity and it is claimed to be a potential drug in the treatment of thrombosis (Lewis et al., 1978) (Healy et al., 1999).

What is more, *A. mellea* as a member of *white-rot fungi* group is capable of lignin degradation, that occurs with the use of several redox enzymes such as lignin peroxidases (LiP), Mn-dependant peroxidases (MnP), versatile peroxidases (VP) and other peroxidases, laccases and tyrosinases (Singh, 2006a).

Mineral compounds and vitamins

The high content of mineral compounds of higher fungi is a well-known fact. Though they constitute a rich source of sufficient minerals, they also accumulate heavy metals and radioactive elements, especially when growing in polluted areas next to the highways (Svoboda et al., 2006) (Kemp, 2002). The level of well assimilable mineral constituents is dependent on many factors, but mostly on the age of fungi, substratum and the diameter of a cap as the highest concentrations of all these compounds and elements can be found within a fungus' pileus (Przybyłowicz and Donoghue, 1988) (Muszynska et al., 2011).

The studies conducted on *A. mellea*'s fruiting bodies collected in Poland revealed the presence of such elements as Na, K, Mg, Ca, Fe, Mn, Zn, Pb, Cu, Cd, Hg, Ag, and Se (Falandysz et al., 2002) (Falandysz et al., 1992).

The vitamin content is extremely valuable since the vitamins play an important role in the human and animal organisms and fungi are reported to be one of the best sources of vitamins, especially vitamin B (Furlani and Godoy, 2008) (Mattila et al., 2001). Research on *A. mellea* showed that comparing to other *Basidiomycetes* it contains a great amount of niacin and vitamins B1, however, the vitamin's B2 content was lower in the comparison to other fungi (Majdańska, 2007) (Podlewska, 2006).

Ecological aspects

Armillaria mellea is considered to be one of the most common and dangerous species of fungal root pathogens worldwide (Baumgartner et al., 2011) (Mańka, 1953). For a long time, it has been recognized as an important plant parasite mostly attacking trees, but also affecting fruit and nut crops resulting in root rot. It can also colonize several orchids, however, in such cases, the orchid is considered a parasite and the host is the mushroom. *A. mellea* attacks both deciduous and coniferous trees of all ages and class range (Sierota, 2001) (Lech and Sierota, 2000) and is considered to have the widest host range among all the *Armillaria* genus, though the ability of spread by means of rhizomorphs is not that well developed as observed in a different *Armillaria* species (e.g. *A. bulbosa*) (Rishbeth, 1982).

Yet there is no effective way of dealing with *Armillaria* root disease. Although the researchers from all over the world work on developing a mean of controlling it, the task is complex because the mushroom feeds itself not only with trees it kills but also other stumps of trees which have died naturally or killed by different factors. Another issue is that the mycelium can survive saprophytically for years in the soil within woody residual roots even after clearing the remains of infected trees and so it contributes as inoculum for infection of the next crop (Redfern and Filip, 1991) (Baumgartner et al., 2011).

As mentioned earlier *A. mellea* can act as a parasite, saprophyte or in a mycorrhizal relationship.

Armillaria mellea as a pathogen

The pathogenic mechanism of *Armillaria mellea*'s action can be dual- it can occur as a parasite colonizing the cambium of living roots or as a saprophyte it can feed on the dead tissue of defeated trees (Hasegawa et al., 2010) (Baumgartner et al., 2011). Most of the *Armillaria* species are reported to be facultative necrotrophs, which means that within their life cycle they become both. In the literature, it has been described as going through two phases, firstly through the parasitic phase including colonizing of living roots. The phase is finished once the fungus kills the cambium and shortly after the saprophytic phase begins in which the fungus utilizes the dead tissue using it as nourishment (Rishbeth, 1985).

A. mellea invades already injured and weakened trees by entering through the wound but also has an ability to penetrate the root without the presence of any damages. The *Armillaria* infection can occur in three ways. The most commonly investigated infections due to their dominant occurrence were infections via mycelium and rhizomorphs, the short-range means of dispersal.

A. mellea uses its rhizomorphs that can attach themselves to the root and can go through the corky layer that protects the root. Firstly the entrance is conducted by mechanical force applied by the tip of a rhizomorph, the host cells being slightly pushed and compressed without a damage (Zelter, 1926). Only later the tip of the penetrating branch secretes enzymes that act upon root bark degrading them and when the penetration is completed it only takes time for the fungus to invade the host and take it over (Harold, 1934).

While the rhizomorphs grow through soil producing wide and long branches that are capable of spreading the infection between diseased and healthy trees without any direct contact between them, the mycelium grows by direct contact with uninfected trees (Redfem and Filip, 1991). The mycelium of *Armillaria* develops not only in tree roots but also spreads along the tree trunk reaching even up to a few metres high (Mańka, 1998).

The last mean of infection that is the least mentioned in the literature is a basidiospore-based infection. This occurs when the basidiospores are being released from the basidiocarp and wind-dispersed to new uninfected areas. Basidiospores seem to be an ideal tool to widespread the infection through the greater distance but due to the difficulties with stumps colonizing they are not and this kind of infections happens quite rarely (Rishbeth, 1985).

Armillaria mellea in a mycorrhizal relationship

Armillaria mellea is capable of performing a quite different and unique kind of plant symbiosis which is known as myco-heterotrophy, a relationship between the majority of plants and some fungi (*Basidiomycota, Ascomycota, Glomeromycota*), when the fungi host plants (Merckx et al., 2009). This type of symbiosis is exhibited by *A. mellea* with the orchids *Galeola* and *Gastrodia* and in this rare case, the plants are considered to be parasitizing the fungus (Kikuchi et al., 2008). The orchid benefits from the mushroom by drawing a carbon from the mycelium and also it lacks chlorophyll, however it has been not reported for the orchid to develop any root disease while being in a mycorrhizal symbiosis with the fungus hence it is supposed that *A. mellea* does not use the orchid as a source of nourishment. It might be that besides that the mushroom is involved too in a typical pathogenic relationship with a second host plant and feeds itself by remaining consolidated with it using its rhizomorphs (Baumgartner et al., 2011) however this interaction with respect to the fungus is yet poorly understood (Cha and Igarashi, 1995b) (Sekizaki et al., 2008).

White-rot fungi's decomposing abilities

As mentioned earlier *Armillaria mellea*'s ability to degrade root bark is achieved by applying mechanical force but mostly by secreting specific enzymes. These powerful extracellular oxidative ligninolytic enzymes digest any wooden material by acting upon main structural components of it, lignin and cellulose (Zhang et al., 2016) They first decompose the cambium underlying the root bark and then xylem (Misiek and Hoffmeister, 2008). This ability is characteristic for all the mushrooms within the *white-rot fungi* group, however, white rotters of the *Basidiomycetes* and *Ascomycetes* class are unique to break down lignin completely, leaving the cellulose mostly intact, hence the white stains on the wood can be noticed.

It was documented that the same digestive enzymes of white rotters that decompose lignin and cellulose are also effective in breaking down a surprisingly wide range of recalcitrant compounds which are held together by the same chemical bonds that are these present in wood, hydrogen-carbon bonds. The mentioned toxins arise from a variety of industrial operations, petroleum being released into environment and tools used for plant protection and they include polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), polychlorinated phenols, organophosphorus compounds and neuroparalytic VX gasses (Turło and Turło, 2013).

Enzymes secreted by this group of fungi extracellularly include lignin peroxidases, manganese-dependent peroxidases, pectin lyases, pectin methylesterases, polygalacturonases, phenol oxidases, proteinases and metalloproteinases, tyrosinases and laccases (Barry et al., 1981) (Lee et al., 2005) (Mwenje and Ride, 1997) (Mwenje and Ride, 1999) (Robene-Soustrade et al., 1992). Manganese-dependent peroxidases along with laccases are documented to play a critical role in lignin degradation, what is more, manganese-dependant peroxidases are enzymes that only *white-rot fungi* can synthesise (Baldrian, 2006) (Schliephake et al., 2003). This complex mix of enzymes allows the fungi to disassemble several materials produced by humans or by nature that are resistant to other ways of degradation and this leads to using white rotters as a tool in mycoremediation.

Mycoremediation

The Environmental Protection Agency gives the definition according to which mycoremediation is a form of bioremediation that uses conditioned native fungi or fungal mycelium to remove and degrade contaminants, to break down hazardous long-chained compounds into less toxic or non-toxic ones (Turło and Turło, 2013). Mycoremediation also holds promise to be capable of removing heavy metals from the environment. The process includes applying mycelium to the contaminated soil, placing mycelial mats over toxic sites or a combination of both techniques (Stamets, 2005). The technologies the mycoremediation uses can be divided into *in situ* methods that treat the contaminated material at the site or *ex situ* methods which involve removing the material and treat it in a specific apparatus used for the cultivation of fungi, a bioreactor (Turło and Turło, 2013). There are three phases of strategies that are anticipated for the successful implementation of mycoremediation with use of *white-rot fungi* and these are inoculum preparation techniques (the first phase), preparing clear technical protocols for the final design and associated engineering process (the second phase), preparation of the remediation protocols for the monitoring, adjustment, continuity and maintenance of the engineering system (the third phase) (Singh, 2006b). Since *A. mellea* remain a member of *white-rot fungi* group within the *Basidiomycota* class it is believed that the species could be successfully applied to mycoremediation technique.

Conclusion

Armillaria mellea is considered as a commonly known fungi species, edible and spread worldwide, nevertheless its various properties and applications could surprise many. As literature shows, there have been plenty of studies regarding species description, covering such topics as morphology, habitat, life cycle and components of the fungus body.

When discovering *A. mellea*'s components, also their medicinal and biological use have been described and applied to number of disciplines or disease entities with positive effects, showing that the world of fungi is offering not only culinary sensations or purely scientific results, but also treatment solutions.

Recently, scientists are focused on investigating a new application for fungi which has been called mycoremediation. This form of bioremediation uses *Basidiomycota* fungi, which *A. mellea* belongs to, to deal with environmental pollution issues. The topic is still not explored well enough, but holds a great promise to be applied worldwide in ecology sector and to be the main tool used in environmental protection.

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References

Abomo-Ndongo S, Mohammed C, Guillaumin JJ (1997). Sexual behaviour of *Armillaria heimii* and *A. mellea* isolates. Africa. For. Pathol. *27*: 207–224.

Amar C, Delaumeny J, Vilkas E (1976). Chemical and biological properties of peptido-glucan fraction from *Armillaria mellea (Basidiomycetes)*. Biochim. Biophys. Acta BBA - Gen. Subj. *421*: 263–271.

Baldrian P (2006). Fungal laccases – occurrence and properties. FEMS Microbiol. Rev. *30*: 215–242.

Barry FP, Doonan S, Ross CA (1981). Cleavage by trypsin and by the proteinase from *Armillaria mellea* at ε- N -formyl-lysine residues. Biochem. J. *193*: 737–742.

Baumgartner K., Coetzee M, Hoffmeister D (2011). Secrets of the subterranean pathosystem of *Armillaria*. Mol. Plant Pathol. *12*: 515–534.

Bermudes D, Petersen R, Nealson K (1992). Low-level bioluminescence detected in *Mycena haematopus* basidiocarps. Mycologia. *84*: 799.

Bernaś E, Jaworska G, Lisiewska, Z (2006). Edible mushrooms as a source of valuable nutritive constituents. Acta Sci. Pol. *5*: 5–20.

Bérubé J, Dessureault M (1989). Morphological studies of the *Armillaria mellea* complex: two new species, *A. gemina* and *A. calvescens*. Mycologia. *81*: 216–225.

Bobek P, Ozdin L, Kuniak L (1995). The effect of oyster mushroom (*Pleurotus ostreatus*), its ethanolic extract and extraction residues on cholesterol levels in serum, lipoproteins and liver of rat. Food Nahr. *39*: 98–99.

Cha JY, Igarashi T (1995a). A note on *Armillaria mellea subsp. nipponica subsp. nov.* in Japan. Mycoscience. *36*: 143–146.

Cha JY, Igarashi T (1995b). *Armillaria* species associated with *Gastrodia elata* in Japan. For. Pathol. *25*: 319–326.

Cha JY, Sung JM, Igarashi, T (1994). Biological species and morphological characteristics of *Armillaria mellea* complex in Hokkaido: *A. sinapina* and two new species, *A. jezoensis* and *A. singula*. Mycoscience. *35*: 39–47.

Chang ST, Wasser SP (2012). The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. Int. J. Med. Mushrooms. *14*: 95–134.

Chattopadhyay A, Rukmini R, Mukherjee S (1996). Photophysics of a neurotransmitter: Ionization and spectroscopic properties of serotonin. Biophys. J. *71*: 1952–1960.

Chi CW, Chen CC, Chen YJ (2013). Therapeutic and radiosensitizing effects of armillaridin on human esophageal cancer cells. Evid. Based Complement. Alternat. Med. *2013*: 1–8.

Cox KD, Scherm H, Riley MB (2006). Characterization of *Armillaria spp.* from peach orchards in the southeastern United States using fatty acid methyl ester profiling. Mycol. Res. *110*: 414–422.

Desjardin DE, Oliveira AG, Stevani, CV (2008). Fungi bioluminescence revisited. Photochem. Photobiol. Sci. *7*: 170.

Donnelly D, Abe F, Coveney D, Fukuda N, O'Reilly J, Polonsky J, Prangé T (1985). Antibacterial sesquiterpene aryl esters from *Armillaria mellea*. J. Nat. Prod. *48*: 10–16.

Falandysz J, Sicińska B, Bona H, Kohonke D (1992). Metals in Honey mushroom *Armilariella mellea*. Bromatol Toxycol Chem. *25*: 171–176.

Falandysz J, Gucia M, Skwarzec B, Frankowska A, Klawikowska K (2002). Total mercury in mushrooms and underlying soil substrate from the Borecka Forest, Northeastern Poland. Arch. Environ. Contam. Toxicol. *42*: 145–154.

Florczak J, Lasota W (1995). Wchłaniane i wiązanie kadmu przez *boczniaka ostrogowatego* w warunkach uprawy. Bromatol Chem Toksykol. *28*: 17–23.

Florczak J, Karmańska A,Wędzisz A (2004). Comparison of the chemical contents of selected wild growing mushrooms. Bromatol Toxykol Chem. *37*: 365–371.

Furlani RP, Godoy HT (2008). Vitamins B1 and B2 contents in cultivated mushrooms. Food Chem. *106*: 816–819.

Gao JM, Yang X, Wang CY, Liu JK (2001). Armillaramide, a new sphingolipid from the fungus *Armillaria mellea*. Fitoterapia. *72*: 858–864.

Gao LW, Li WY, Zhao YL, Wang JW (2009). The cultivation, bioactive components and pharmacological effects of *Armillaria mellea*. Afr. J. Biotechnol. Vic. Isl. *8*: 7383–7390.

Gębska AK (2009). Wpływ selenu na własności antyoksydacyjne grzybni *Armillaria mellea*. Warszawski Uniwersytet Medyczny. Praca dyplomowa.

Gumińska B, Wojewoda W (1988). Grzyby i ich oznaczanie. Warszawa: Państwowe Wydawnictwo Rolnicze i Leśne.

Harold TE (1934). Studies on *Armillaria melea* (Vahl) Quel., Infection, Parasitism And Host Resistance. J. Agric. Res. *48*: 187–218.

Hasegawa E, Ota Y, Hattori T, Sahashi N, Kikuchi T (2010). Ecology of *Armillaria* species on conifers in Japan. For. Pathol. *41*: 429–437.

Healy V, O'Connell J, McCarthy TV, Doonan S (1999). The Lysine-Specific Proteinase from *Armillaria mellea* is a member of a novel class of Metalloendopeptidases located in *Basidiomycetes*. Biochem. Biophys. Res. Commun. *262*: 60–63.

Isbister GK, Bowe SJ, Dawson A, Whyte IM (2004). Relative toxicity of selective serotonin reuptake inhibitors (SSRIs) in overdose. J. Toxicol. Clin. Toxicol. *42*: 277–285.

Kabulska, A.P. (2015). Biosynteza, izolacja i badanie struktury analogu lentinanu - polisacharydowego leku z grupy modyfikatorów odpowiedzi biologicznej. Warszawski Uniwersytet Medyczny. Praca dyplomowa.

Kalač P (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chem. *113*: 9–16.

Karkocha I (1964). Badania wartości odżywczej *gąski zielonej* (*Tricholoma equestre*) i *opieńki miodowej* (*Armillaria mellea*). Rocz. PZH. *15:* 311–315.

Kemp K (2002). Heavy metals in edible mushrooms. Int. J. PIXE. *12*: 117–124.

Kikuchi G, Higuchi M, Yoshimura H, Morota T, Suzuki A (2008). In vitro symbiosis between *Gastrodia elata* Blume (*Orchidaceae*) and *Armillaria* (*Tricholomataceae*) species isolates from the orchid tuber. J Jpn Bot. *83*: 77–87.

Kim SK, Im J, Yun CH, Son JY, Son CG, Park DK, Han SH (2008). *Armillariella mellea* induces maturation of human dendritic cells without induction of cytokine expression. J. Ethnopharmacol. *119*: 153–159.

Korhonen K, Hintikka V (1974). Cytological evidence for somatic diploidization in dikaryotic cells of *Armillariella mellea*. Arch. Microbiol. *95*: 187–192.

Lamour A, Termorshuizen AJ, Volker D, Jeger MJ (2007). Network formation by rhizomorphs of *Armillaria lutea* in natural soil: Their description and ecological significance. FEMS Microbiol. Ecol. *62*: 222–232.

Lech P, Sierota Z (2000). Poziom zagrożenia lasów grzybami patogenicznymi. In: Stan uszkodzenia lasów w Polsce w 1999 roku na podstawie badań monitoringowych. Warszawa: Biblioteka Monitoringu Środowiska; 59–65.

Lee SY, Kim JS, Sapkota K, Shen MH, Kim S, Chun HS, Yoo JC, Choi HS, Kim MK (2005). Purification and characterization of fibrinolytic enzyme from cultured mycelia of *Armillaria mellea*. Protein Expr. Purif. *43*: 10–17.

Lewis WG, Basford JM, Walton PL (1978). Specificity and inhibition studies of *Armillaria mellea* protease. Biochim. Biophys. Acta BBA - Enzymol. *522*: 551–560.

Lisiewska M, Szmid M (1972). Przewodnik grzyboznawczy. Poznań: PWN.

Majdańska M (2007). Badanie właściwości redukcyjnych i antyoksydacyjnych wyciągów wodnych i metanolowych otrzymanych z grzybni *Armillaria mellea* nieselenowanej i selenowanej. Warszawski Uniwersytet Medyczny. Praca dyplomowa.

Mańka K (1953). Badania terenowe i laboratoryjne nad *opieńką miodową- Armillaria mellea* (Vahl) Quel. Pr. Inst Bad Śn. *94*: 1–96.

Mańka K (1998). Fitopatologia leśna. Warszawa: PWRiL.

Marshall NL (2003). Mushroom book. Montana: Kessinger Publishing.

Mattila P, Könkö K, Eurola M, Pihlava JM, Astola J, Vahteristo L, Hietaniemi V, Kumpulainen J, Valtonen M, Piironen V (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. J. Agric. Food Chem. *49*: 2343–2348.

Merckx V, Bidartondo MI, Hynson NA (2009). Myco-heterotrophy: When fungi host plants. Ann. Bot. *104*: 1255–1261.

Midland SL, Izac RR, Wing RM, Zaki AI, Munnecke DE, Sims, JJ (1982). Melleolide, a new antibiotic from *Armillaria mellea*. Tetrahedron Lett. *23*: 2515–2518.

Mihail JD (2015). Bioluminescence patterns among north American *Armillaria* species. Fungal Biol. *119*: 528–537.

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SZKOŁA

KRÓTKO

Mihail JD, Bruhn JN (2007). Dynamics of bioluminescence by *Armillaria gallica, A. mellea* and *A. tabescens*. Mycologia. *99*: 341–350.
Misiek M, Hoffmeister D (2008). Processing sites involved in intron splicing of *Armillaria* natural product genes. Mycol. Res. *112*: 216–224.
Murrill W (1915). Luminescence in the fungi. In: Mycologia. *7*: 131–133.
Muszyńska B, Maślanka A, Ekiert H, Sułkowska-Ziaja K (2001). Analysis of indole compounds in *Armillaria melea* fruiting bodies. Acta Pol. Pharm. *68*: 93–97.
Muszyńska B, Ziaja K, Ekiert H (2009). Indole compounds in fruiting bodies of some selected *Macromycetes* species and their mycelia cultured in vitro. Pharmazie. *64*: 479–480.
Muszyńska B, Sułkowska-Ziaja K, Ekiert H (2011). Indole compounds in fruiting bodies of some edible *Basidiomycota* species. Food Chem. *125*: 1306–1308.
Mwenje E, Ride JP (1997). The use of pectic enzymes in the characterization of *Armillaria* isolates from Africa. Plant Pathol. *46*: 341–354.
Mwenje E, Ride JP (1999). Purification and characterization of an endo-polygalacturonase (PG1) from a zimbabwean species of *Armillaria*. Physiol. Mol. Plant Pathol. *55*: 131–139.
Nowak M (2005). Optymalizacja warunków wzrostu *opieńki miodowej* oraz porównanie składu uzyskanej grzybni i owocników pochodzących ze środowiska naturalnego. Uniwersytet Medyczny w Warszawie. Praca dyplomowa.
O'Reilly P (2011). Fascinated by fungi: Exploring the history, mystery, facts, and fiction of the underworld kingdom of mushrooms. United Kingdom: First Nature.
Ouzouni PK, Petridis D, Koller WD, Riganakos KA (2009). Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. Food Chem. *115*: 1575–1580.
Phan CW, David P, Naidu M, Wong KH, Sabaratnam V (2014). Therapeutic potential of culinary-medicinal mushrooms for the management of neurodegenerative diseases: Diversity, metabolite, and mechanism. Crit. Rev. Biotechnol. *35*: 355–368.
Pochanavanich P, Suntornsuk W (2002). Fungal chitosan production and its characterization. Lett. Appl. Microbiol. *35*: 17–21.
Podlewska A (2006). Porównanie właściwości biobójczych wyciągów alkoholowych i wodnych otrzymanych z grzybni *Armillaria mellea*. Uniwesytet Medyczny w Warszawie. Praca dyplomowa.
Przybyłowicz P, Donoghue J (1988). Nutritional and health aspects of shiitake. In: Shiitake Growers Handbook. Dubuque, IA: Kendall Hunt Publishing Co.
Redfem DB, Filip GM (1991). Inoculum and Infection. In: Agricultural Handbook. Washington D.C.: USDA Forest Service; 48–61.
Rishbeth J (1982). Species of *Armillaria* in southern England. Plant Pathol. *31*: 9–17.
Rishbeth J (1985). Infection cycle of *Armillaria* and host response. For. Pathol. *15*: 332–341.
Robene-Soustrade I, Lung-Escarmant B, Bono JJ, Taris B (1992). Identification and partial characterization of an extracellular manganese-dependent peroxidase in *Armillaria ostoyae* and *Armillaria mellea*. For. Pathol. *22*: 227–236.
Schliephake K, Baker WL, Longergan GT (2003). Decolorization of industrial wastes and degradation of dye water. In: Fungal Biotechnology in Agricultural, Food and Environmental Applications. New York: Marcel Dekker.
Sekizaki H, Kuninaga S, Yamamoto M, Asazu SN, Sawa S, Kojoma M, Yokosawa R, Yoshida N (2008). Identification of *Armillaria nabsnona* in *Gastrodia* tubers. Biol. Pharm. Bull. *31*: 1410–1414.
Sierota Z (2001). Choroby lasu. Warszawa: Centrum Informacyjne Lasów Państwowych.
Singh H (2006a). Fungal metabolism of polycyclic aromatic hydrocarbons. In: Mycoremediation: Fungal Bioremediation. New York, NY, United States: Wiley-Interscience; 283–356.
Singh H (2006b). Mycoremediation: Fungal Bioremediation. In: Mycoremediation. New Jersey: Wiley-Interscience; 17–18.
Stamets P (2005). Mycoremediation. In: Mycelium Running. How Mushrooms Can Help Save the World. New York: Ten Speed Press; 86–113.
Stasiak AA (2008). Badanie właściwości antyoksydacyjnych i stopnia zmiatania wolnych rodników wyciągów metanolowych i wodnych otrzymanych z grzybni nieselenowanej i selenowanej *Armillaria mellea*. Warszawski Uniwersytet Medyczny. Praca dyplomowa.
Stone TW, Mackay GM, Forrest CM, Clark CJ, Darlington LG (2003). Tryptophan metabolites and brain disorders. Clin. Chem. Lab. Med. 41: 852-859.
Strapáč I, Baranová M, Smrčová M, Bedlovičová Z (2016). Antioxidant activity of honey mushrooms (*Armillaria mellea*). Folia Vet. *60*: 37-41.
Sułkowska-Ziaja K, Muszyńska B, Końska G (2005). Biologically active compounds of fungal origin displaying antitumor activity. Acta Pol Pharm. 62: 153-159.
Svoboda L, Havlíčková B, Kalač P (2006). Contents of cadmium, mercury and lead in edible mushrooms growing in a historical silver-mining area. Food Chem. *96*: 580–585.
Turło J, Turło A (2013). Application of mushroom cultures and isolated enzymes for biodegradation of organic environmental pollutants. Mil. Pharm. Med. *3*: 27–35.
Vaz JA, Barros L, Martins A, Santos-Buelga C, Vasconcelos MH, Ferreira IC (2001). Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions. Food Chem. *126*: 610–616.
Weitz HJ (2004). Naturally bioluminescent fungi. Mycologist. *18*: 4–5.
Weitz HJ, Campbell CD, Killham K (2002). Development of a novel, bioluminescence-based, fungal bioassay for toxicity testing. Environ. Microbiol. *4*: 422–429.
Wilson AM, Wilken PM, van der Nest MA, Steenkamp ET, Wingfield MJ, Wingfield BD (2015). Homothallism: An umbrella term for describing diverse sexual behaviours. IMA Fungus. *6*: 207–214.
Yang J, Yuwu C, Xiaozhang F, Dequan Y, Xiaotian L (1984). Chemical constituents of *Armillaria mellea* mycelium I. Isolation and characterization of Armillarin and Armillaridin. Planta Med. *50*: 288–290.
Yang J, Su Y, Wang Y, Feng X, Yu D, Cong P, Tamai M, Obuchi T, Kondoh H, Liang X (1989). Isolation and structures of two new sesquiterpenoid aromatic esters: Armillarigin and Armillarikin. Planta Med. *55*: 479–481.
Yang J, Su Y, Wang Y, Feng X, Yu D, Liang X (1990). Studies on the chemical constituents of *Armillaria mellea* mycelium. V. Isolation and characterization of Armillarilin and Armillarinin. Acta Pharm. *25*.
Zavastin D, Mircea C, Aprotosoaie AC, Miron A (2015). *Armillaria mellea*: phenolic content, in vitro antioxidant and antihyperglycemic effects. Med Chir Soc Med Nat. *119*.
Zelter SM (1926). Observations on infections of apple and prune roots by *Armillaria mellea* Vahl. Phytopathology. *16:* 479-484.
Zhang L, You T, Zhou T, Xu F (2016). Synergistic effect of white-rot fungi and alkaline pretreatments for improving enzymatic hydrolysis of poplar wood. Ind. Crops Prod. *86*: 155–162.
Źródłowski Z (1995). The influence of washing and peeling of mushrooms *Agaricus bisporus* on the level of heavy metal contaminations. Pol J Food Nutr Sci. *4/45*: 26–33.

Przegląd właściwości biologicznych, chemicznych i ekologicznych gatunku Opieńka miodowa (łac. *Armillaria mellea*)

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Opieńka miodowa (łac. *Armillaria mellea*) jest powszechnie występującym gatunkiem grzyba, który przynależy do typu Podstawczaków. Opieńka jest jadalna- jej owocniki i ryzomorfy są bogatym źródłem polisacharydów, białek, tłuszczów oraz innych makroelemntów. Gatunek znalazł również zastosowanie w medycynie, jego właściwości lecznicze zostały przebadane i opisane w wielu publikacjach. Opieńka miodowa posiada wachlarz interesujących właściwości, z czego najbardziej widowiskowymi są bioluminescencja i zdolność do wytwarzania ryzomorf. Ryzomorfy umożliwiają transport związków mineralnych, a także służą jako narzędzie do atakowania i infekowania sąsiadujących roślin, dzięki czemu gatunek jest klasyfikowany jako niebezpieczny patogen roślin. Opieńka miodowa jest wyposażona w enzymatyczny kompleks ligninolityczny, który odgrywa kluczową role w degradacji drewna i jest cechą charakterystyczną gatunków należących do grupy grzybów zwaną w języku angielskim *white-rot fungi*, której opieńka jest członkiem. W związku z tym, grzyby z tej grupy okazały się być także zdolne do degradacji wielu niebezpiecznych, zanieczyszczeń środowiska, co pokazuje zasadność użycia grzybów *white-rot* w mykoremediacji.

Key words: *Armillaria mellea*; Opieńka miodowa; Ryzomorfy; Bioluminescencja; Medyczne grzyby; Patogen roślin, White-rot fungi; Mykoremediacja

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