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Mycelium Growth and Biological Efficiency of *Ganoderma lucidum* on Substrate Supplemented with Different Organic Additives

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Ganoderma lucidum (W. Curt.: Fr) P. Karst. is a mushroom exhibiting various medicinal properties, popular particularly in Asia. It is grown on a substrate based on hardwood sawdust. Other organic materials, usually agricultural or industrial waste supplemented with various additives, are also used in the cultivation of this mushroom. Numerous studies have shown that the composition of the substrate has a significant effect on mycelium growth and biological efficiency of Reishi mushroom. The presented analysis determined the effect of different organic substances on mycelium growth and biological efficiency of several *G. lucidum* isolates (Gan 18, Gan Li 27/3, Gan 7, Gan 112) obtained from mushrooms growing in the wild and from strains of this mushroom (GL 01, GL 02, GL 03 and GL 04). Growing substrate containing oak sawdust supplemented with wheat bran (20%), rye grain (25%), ground soy (7%), ground rapeseed (10%) or meat–and–bone meal (10%). These additives had a considerable effect on mycelium growth and its biological efficiency. A different response to sawdust substrate additives was found for the group of isolates and strains of *G. lucidum*. All the additives, except for meat–and–bone meal, had a positive effect on mycelium growth and its biological efficiency. In the case of *G. lucidum* strains a more rapid mycelium growth and a greater biological efficiency were observed for its isolates collected from nature, irrespective of the type of substrate additive.

Key words: *Ganoderma lucidum*, agro–industrial residues, mycelium growth, biological efficiency

INTRODUCTION

Ganoderma lucidum (W. Curt.: Fr.) P. Karst. (in China known as Ling Zhi and in Japan as Reishi) is one of the most popular medicinal mushrooms in China, Japan, Korea and other Asian countries (Stamets, 2000; Chang and Miles, 2004; Zhou *et al*., 2012). Numerous studies have shown that Reishi mushroom contains several pharmacologically active substances (Wasser, 2005, 2011; Paterson, 2006; Boh *et al*., 2007). Medicinal properties of *G. lucidum* are connected first of all with its anti–cancer and immunomodulatory effects (Wasser, 2002; Sanodiya *et al*., 2009; Xu *et al*., 2011; Batra *et al*., 2013). Numerous preparations based on *G. lucidum* are commercially available worldwide and the observed demand for these products shows a marked upward trend (Lai *et al*., 2004).

Ganoderma lucidum grows on dead trees of many species, mainly deciduous (hardwood), while it is rather rarely found in coniferous (softwood) species (Stamets, 2000; Chen, 2002; Wasser, 2005). As it was reported by Curvetto *et al*. (2002), Hsieh and Yang (2004) and Sanodiya *et al*. (2009), in order to meet the increasing demand this species is grown on different natural solid substrates as well as liquid nutrient media. Fruit bodies of *G. lucidum* are traditionally produced on wood, sawdust and grain (Stamets, 2000; Chen, 2002 2004; Wasser, 2005; Boh *et al*., 2007; Azizi *et al*., 2012). As it was reported by Sukarno *et al*. (2009), hardwood is particularly useful in this case. In the culture of *G. lucidum* other organic materials are also used, most frequently agricultural or industrial waste, *e.g*. sunflower husks (Gonzalez–Matute *et al*., 2002), coir (Mishra and Singh, 2008), different types of straw (Mishra and Singh, 2008; Ke *et al*., 2011; Veena and Pandey, 2011) as well as hemp and flax shives (Siwulski *et al*., 2010). Numerous studies have shown that the application of such substrate additives as wheat, rice or maize bran (Chen, 2004; Erkel, 2009a; Azizi *et al*., 2012), corn meal and gram flour (Gurung *et al*., 2012), tea waste (Peksen and Yakupoglu, 2009), soy residue (Hsieh and Yang, 2004), molasses (Stamets, 2000; Erkel 2009b), malt (Gonzalez–Matute *et al*., 2002), stillage grain (Yang *et al*., 2003), yeasts (Ke *et al*., 2011), food waste compost (Jo *et al*., 2013a,b) and fishing industry waste (Lakshmi, 2013) may improve yields of Reishi mushrooms. The effect of these additives on yielding depends first of all on their contents of nitrogen sources and the supply of carbon compounds available for mycelium (Hsieh and Yang, 2004; Erkel, 2009b; Peksen and Yakupoglu, 2009; Jo *et al*., 2013a).

Many studies showed an effect of cultivation conditions and the composition of the substrate on mycelium growth, biological efficiency and contents of bioactive compounds in Reishi mushrooms (Siwulski and

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Sobieralski, 2001; Hsieh and Yang, 2004; Rai *et al*., 2004; Sobieralski and Grzebielucha, 2005, 2006a,b; Boh *et al*., 2007; Mishra and Singh, 2008; Erkel 2009a; Peksen and Yakupoglu, 2009; Siwulski *et al*., 2010; Skalicka–Woźniak *et al*., 2012). Philippoussis *et al*. (2001), Peksen and Yakupoglu (2009) as well as Fanadzo *et al*. (2010) showed an effect of physical and chemical properties of the cultivation substrate on the yielding level and biological efficiency in many fungal species. The period of substrate overgrowth is also a very important indicator, since to a considerable extent it determines the economic effects of culture. Substrates, which are slowly overgrown by mycelium, are susceptible to fungal and bacterial infections and this results in a decreased yielding (Philippoussis *et al*., 2001).

The aim of the presented study was to determine the effect of different organic additives on mycelium growth and biological efficiency of selected wild *G. lucidum* isolates and the culture collection strains.

MATERIAL AND METHODS

The analyses were conducted on four isolates (Gan 18, Gan Li 27/3, Gan 7 and Gan 112) and four strains of *G. lucidum* (GL 01, GL 02, GL 03 and GL 04) coming from the collection of cultivated and medicinal fungi of the Department of Vegetable Crops, the Poznan University of Life Sciences.

Mycelium growth and biological efficiency of *G. lucidum* were tested on solid substrate containing *Q. robur* L. sawdust (the 0.5–3 mm fraction at 80%, the 0.3–0.4 mm fraction at 10%, <0.3 mm at 10%), which were supplemented with 20% wheat bran, 25% rye grain (cv. 'Słowiańskie'), 7% ground soy, 10% ground rapeseed or 6% meat–and–bone meal (w/w). All additives came from MŁYNPASZ Sp. z o.o,, Poland. At the application of the above mentioned amounts of additives the substrates contained a comparable amount of protein, *i.e*. 3.2 g per 100 g d.m. substrate. The amount of protein in additives was calculated on the basis of the analyses of total nitrogen contents according to Kjeldahl using the N x 6.25 conversion factor. The control comprised oak sawdust substrate with no additives. After all the components in the substrates were mixed in, moisture content was determined by the gravimetric method (105°C). Based on the known water contents in the mixtures they were moistened with tap water until 60% moisture content was obtained.

Mycelium growth

Glass test tubes of 1.5 cm in diameter and 16 cm in length were filled with previously produced substrates to the height of 12 cm and next sealed with cotton wool stops. The test tubes with substrates were sterilized in 121ºC at 0.1 MPa for 1 h. After cooling the substrates to room temperature (ca. 21°C) a 1–cm layer of mycelium on wheat grain was placed on the top of substrate. The incubation process was run for 14 days at 25ºC and 80–85% relative humidity, with no access to light. Mycelium growth was measured accurate to 1 mm after incubation was completed. A measure of growth was determined by the thickness of the substrate layer overgrown by mycelium. The experiment was run in a random design in two series of five replications each.

Biological efficiency

Polypropylene culture bags equipped with a microbiological filter (Fungi Laboratories, Spyra, Poland) were filled with 2.5 kg previously produced substrates and sterilized in 121ºC at 0.1 MPa for 1.5 h. After the substrates cooled to room temperature (ca. 21°C) they were spawned with mycelium on wheat grain at 50 g per bag. Bags were sealed using a sealer (Impuls Sealer KSA–450, Poland), incubated in an incubation chamber (Pol–Eko–Aparatura, ST–500) with no access to light at a temperature of 25ºC and 90–95% relative humidity until the substrate was completely overgrown by mycelium. After complete substrate overgrowth by mycelium the plastic was removed from the bags up to the upper substrate surface. Next bags were transferred to a cultivation chamber, in which the temperature of 23ºC±1° and 85 – 90% relative humidity were maintained, and lighting was provided by Day–Light fluorescent light (40W lamps, Osram, Germany) at 500 lx for 12 h per day. Fruit bodies were harvested as they were growing and maturing, cutting them at the stipe base. Biological efficiency (BE) expressed in (%) was equivalent to the weight of fresh fruit bodies per 100 g substrate dry weight. The experiment was run in a random design in two growing cycles with five replications each.

Statistical analysis

Results were subjected to the analysis of variance for factorial experiments. The significance of differences between the tested factors was determined using the Newman–Keuls test at α =0.05.

RESULTS AND DISCUSSION

Mycelium growth

Statistical analysis of recorded results showed a significant effect of organic additives used in solid substrate with oak sawdust on mycelium growth of isolates and strains of *G. lucidum* (Table 1). It was found that four among the applied agri–food products (wheat bran, rye grain as well as ground soy and ground rapeseed) had an advantageous effect on mycelium growth. In turn, an addition of meat–and–bone meal did not result in increased mycelium growth rates of tested isolates and strains of *G. lucidum*.

Chen (1999) in mycelium of *Ganoderma* growing on oak sawdust substrate reported the best growth of the tested mushroom in the variant with an 18% addition of wheat bran. Rai *et al*. (2004) and Sobieralski and Grzebielucha (2005) reported in their studies that the mycelium growth rate of Reishi mushroom increases with an increase in the amount of bran added to the substrate. However, an addition exceeding 20% reduced mycelium growth rate. For this reason in this study this substance was added at 20% concentration. The observed

Table 1. Mycelium growth of isolates and strains of *Ganoderma lucidum* on oak sawdust substrate supplemented with different organic additives after 14 days of incubation

Isolates/ strains	Organic additives					
	0% control	20% wheat bran	25% rye grain	7% ground soy	10% ground rapeseed	10% meat–bone meal
Gan 18	62	68	56	71	68	53
Gan Li 27/3	42	45	63	52	47	45
Gan 7	52	57	59	55	50	48
Gan 112	30	32	35	38	31	32
GL 01	102	123	128	117	110	107
GL 02	91	109	106	120	117	94
GL 03	98	125	126	130	115	93
GL 04	107	123	133	116	120	109
Mean	73a	85bc	88c	87c	82b	73

Means marked with the same letters do not differ significantly at $\alpha = 0.05$

mycelium growth rate, depending on the applied additive, ranged from 5.2 to 6.3 mm per day. Similar results were described by Jo *et al.* (2013a), as in their study conducted on oak sawdust substrate with a 20% addition of rice bran growth increment was 5.3 mm per day. In turn, in substrates supplemented with food waste compost it ranged from 3.6 to 5.3 mm per day. Similar results (3.2–5.8 mm per day) were recorded also by Yang *et al.* (2003) on substrate from black locust sawdust with an addition of malt (stillage grain), wheat bran and ground rice. In studies conducted by Gonzalez–Matute *et al.* (2002) on substrate from sunflower husks supplemented with wheat bran and malt the growth rate of mycelium ranged from 5.33 to 5.88 mm per day. A much greater growth rate of mycelium was reported by Azizi *et al.* (2012), when comparing 5% and 10% wheat bran additions and 2.5% and 5% additions of maltose recorded the fastest mycelium growth of Reishi mushrooms (10.6 mm per day) on the substrate with an 10% addition of bran and 2.5% addition of maltose. Differences in mycelium growth rates were probably connected with different compositions of substrates used in the experiments and different C/N ratios.

In the presented experiments strains of *G. lucidum* exhibited a much faster growth rate than isolates collected from nature (Fig. 1). The mean mycelium growth rates of isolates on substrates supplemented with organic additives varied greatly and after 14 days of incubation ranged from 33 to 66 mm. In turn, growth rates in strains were very similar (from 106 to 118 mm) and it was only in the GL 02 strain a weaker mycelium growth was observed in comparison to the other strains. The varied mycelium growth in different *Ganoderma* strains were also reported by Siwulski and Sobieralski (2001) and Sobieralski and Grzebielucha (2005). Differences were also found in a study by Sukarno *et al.* (2004), in which commercial mycelium of *G. lucidum* was characterised by a more rapid growth rate than isolates coming from natural localities.

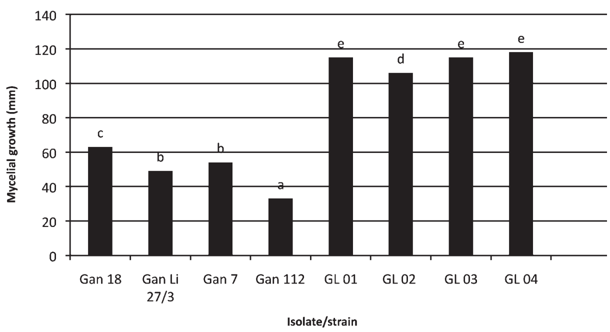

Fig. 1. Mean mycelium growth of isolates and strains of *Ganoderma lucidum* on oak sawdust substrate supplemented with different organic additives.

Biological efficiency

Results of numerous studies show that supplementation of growing substrate containing sawdust has a positive effect on its biological efficiency. It was confirmed in the presented experiments (Table 2). Four out of the applied enriching additives (wheat bran, rye grain, ground soy and ground rapeseed) showed an advantageous effect on biological efficiency of tested isolates and *Ganoderma* strains. At the addition of bone meal to the growing substrate no positive effect was observed on the biological efficiency of this mushroom. Mean biological efficiency, depending on the applied additive, was 10 – 22%. Three of the compared substances exhibited 22% biological efficiency. In turn, biological efficiency on the substrate supplemented with meat–an–bone meal was identical as on the control substrate and amounted to 10%.

According to Chen (2004), in culture on the substrate containing sawdust a 5 – 25% addition of bran was used. In the presented experiments wheat bran was added at 20%. Erkel (2009a), when using a 20% addition of wheat bran to oak sawdust substrate reported a slightly lower biological efficiency of *Ganoderma* (18.6%) than that found in this presented study. Even worse results

Table 2. Biological efficiency of isolates and strains of *Ganoderma lucidum* on oak sawdust substrate supplemented with different organic additives

Isolates/ strains	Organic additives					
	0% control	20% wheat bran	25% rye grain	7% ground soy	10% ground rapeseed	10% meat– bone meal
Gan 18	7	13	15	14	13	8
Gan Li 27/3	6	14	16	14	12	7
Gan 7	6	15	14	15	12	7
Gan 112	8	18	18	19	13	9
GL 01	13	38	40	42	34	17
GL 02	12	28	27	29	23	16
GL 03	10	25	20	23	23	9
GL 04	10	27	23	24	21	10
Mean	9a	22b	22b	22b	22b	10a

Means marked with the same letters do not differ significantly at α =0.05

were recorded by that author when using a 20% addition of maize and rice bran, as biological efficiency in those cases was 16.3% and 13.56%. The same author (Erkel 2009b) on poplar sawdust substrate with a 20% addition of wheat bran obtained 17.2% efficiency. In a study by Azizi *et al*. (2012) biological efficiency of Reishi mushrooms grown on poplar sawdust substrate with a 10% addition of wheat bran and a 5% addition of maltose extract was higher, amounting to 18.68%. This could have been caused by an addition of maltose extract or the property of the tested fungal strain.

Peksen and Yakupoglu (2009), when growing two strains of *Ganoderma* on hornbeam sawdust substrate with an 18% addition of wheat bran, depending on the strain obtained biological efficiency ranging from 13 to 20%. Gurung *et al*. (2012), depending on the applied substrate additions of two types of sawdust, recorded from 6.9% to 12.19% efficiency. Those authors recorded the highest biological efficiency (22.62%) for the substrate of sawdust from Nepalese alder (*Alnus nepalensi*) with a 10% addition of gram flour. In turn, a 10% addition of wheat bran resulted in a slightly lower biological efficiency of Reishi mushrooms (14.58%).

Stamets (2000), in cultivation of *G. lucidum* recommends a maximum 15% addition of rice bran to the substrate. Mishra and Singh (2008) investigated the effect of wheat and rice bran (2.5% and 5%) on yielding of three Reishi isolates grown on substrates from different types of agricultural waste (coir, wheat straw, chickpea straw and mustard straw). The best results were recorded for a 5% rice bran addition to wheat straw substrate. Biological efficiency in the experiments conducted by those authors ranged from 10% to 17% and it was lower than that presented in this study (22%). For the addition of rice bran (5 and 10%) to the substrate containing wheat straw the efficiency recorded for different *Ganoderma* isolates efficiency ranged from 5.85 to 20% (Mishra and Singh, 2012). A much greater biological efficiency (29.9%) after the application of a 10% addition of rice bran was recorded by Veena and Pandey (2011). Results reported by those authors could have been influenced by the different composition of the substrate used in their experiments.

Supplementation of plant residue, i.e. sugar cane, coir and chips, with fish processing waste made it possible to obtain biological efficiency of Reishi mushrooms at 0.92–12.95%. Increasing the level of fish waste in the growing substrate resulted in the efficiency of 0.92–4.63%, while an addition of meat–and–bone meal (10%) provided 10% efficiency (Lakshimi, 2013).

Most additives used in the presented study in oak sawdust substrate resulted in mean biological efficiency of 22%, which indicates very good properties of these materials in growing Reishi musrooms. The highest biological efficiency (42%) among the tested isolates and strains of *Ganoderma* was recorded for the GL01 strain grown on substrate supplemented with ground soy.

In the presented study strains of *G. lucidum* exhibited much greater biological efficiency that the tested isolates (Fig. 2). The greatest biological efficiency (37%) was recorded for the GL01 strain, while it was lowest (14%) for isolates Gan 18, Gan Li 27/3 and Gan 7. In a study by Peksen and Yakupoglu (2009), mean biological

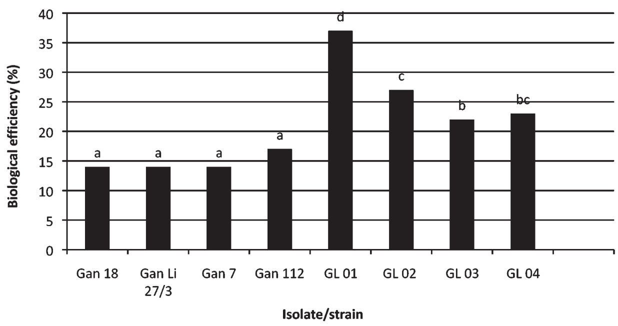

Fig. 2. Mean biological efficiency of isolates and strains of *Ganoderma lucidum* on oak sawdust substrate supplemented with different organic additives.

efficiency of the two compared strains of *Ganoderma* did not differ significantly and in both cases was approx. 24%.

Conducted experiments confirmed that organic waste materials may be successfully used in growing Reishi mushrooms and have a significant effect on mycelium growth and their biological efficiency. In the presented study mycelium growth and biological efficiency depended also on the used isolates and strains of Reishi mushrooms.

Results of our experiments and those reported by other researchers concerning mycelium growth and biological efficiency differ considerably depending on the type of used substrate and applied supplements. In most cases supplementation of growing substrates had a positive effect on growing results of *G. lucidum*. This problem needs to be investigated in further research. Obviously the selection of growing substrate and additives will first of all be determined by their availability and price.

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