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Effect of wood residues on the growth of *Ganoderma lucidum*

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Abstract

Sawmill industries generate considerable amounts of low value wood residues. Fungal decomposition of lignocellulosic biomass allows the conversion of wood residues into valuable products. The selection of the most suitable fungal strains and media are essential to optimise the bioconversion of wood residues and serves as a basis for mushroom cultivation industries. The aim of this study was to find the best combinations of *Ganoderma lucidum* strains and substrate media to optimise the cultivation of the fungus. Mycelial growth and culture characteristics of *G. lucidum* isolated from *Betula pubescens* and *Picea abies* in Finland were tested on agar media containing different wood residues. These included *Betula* spp., *Populus tremula*, *Larix* sp., *Pinus sylvestris*, *Alnus incana* and *P. abies* sawdust, which were added to malt extract agar, potato dextrose agar and water agar. The results showed significant differences in the mycelial growth between all interaction levels (agar media, wood species and fungal strain). The addition of malt extract significantly enhanced the growth of the fungus in comparison to potato dextrose or water agar. The wood sawdust

most suitable for mycelial growth was *Betula* spp., followed by *P. tremula*. Strains originally isolated from *P. abies* also presented higher mycelial growth in media with hardwood sawdust. These findings demonstrate that *Betula* spp. and *P. tremula* sawdust stimulate the growth of *G. lucidum*. Thus, it is possible to cultivate the fungus on a variety of wood residues from sawmill industries.

Introduction

The forest industries generate considerable amounts of wood residues in Europe that are mainly used for energy production (Forest Europe 2015). Most of the wood residues generated from sawmill industries are composed of wood chips, bark and sawdust, and are considered low value side streams. The implementation of new bioeconomy standards from the European Commission (European Commission 2012) and the desire of forestry companies to improve their wood residue management have led to initiatives to find innovative applications for these side streams. Wood is a lignocellulosic raw material composed of cellulose, hemicellulose and lignin polymers. The composition and linkages of wood polymers are diverse reflecting the complexity of the wood (Sánchez 2009; Andlar et al. 2018). Despite this, several fungi and other microorganisms can decompose lignocellulosic biomass to obtain carbon and energy resources.

Fungal mechanisms of lignocellulose decomposition are diverse and complex (Andlar et al. 2018). Saprotrophic mushroom species are able to decompose lignocellulosic biomass by producing fungal enzymes, also known as hydrolytic and oxidative enzymes (Sánchez 2009). One of the main difficulties in the process of lignocellulose decomposition is the degradation of lignin. Hence, the cultivation of white-rot fungi is presented as an opportunity for the delignification of lignocellulosic biomass (Andlar et al. 2018). Wood residues generated by the forestry sector have been previously used as substrates for the cultivation of edible and specialty mushrooms. The use of forest industry side streams for the cultivation of specialty mushrooms involves forestry and agricultural practices together and supports the main principles of the circular economy (Grimm & Wösten 2018).

Ganoderma lucidum (W. Curtis) P. Karst. (Basidiomycota) is a white rot fungus that colonises the heartwood and roots of trees. The fungal species hosts are a broad number of deciduous and conifer tree species. In the Nordic countries, the fungus has been previously found evenly in deciduous and conifer tree species (Kajava & Silver 2016). In Finland, a recent field inventory from several biogeographical locations within the country found the fungus growing mostly on *Picea abies* stumps (Veteli et al. 2019). The taxonomy of the *Ganoderma* species is not clear, leading to confusion with the species nomenclature (Cao et al. 2012). Studies based on morphological characteristics and molecular data have pointed out that '*G. lucidum*' from Asia does not correspond with the originally described European species (Moncalvo & Ryvarden 1997; Cao et al. 2012; Wang et al. 2012; Zhang et al. 2017).

Ganoderma lucidum contains several components that are responsible of its bioactive properties, such as antimicrobial and antiviral effects (Yoon et al. 1994; Linnakoski et al. 2018). The quantity and quality of the bioactive components differ between strains, origin, cultivation technique and growing conditions. Moreover, bioactive components are the main factors to consider when defining the market value of the fungus.

Several authors have tested the cultivation of *G. lucidum* on a variety of substrates such as beech, poplar and hornbeam sawdust, among others (Azizi et al. 2012). Moreover, *Ganoderma* spp. have proved to cause extensive delignification in several wood species such as *Quercus* spp., *Pinus taeda* and *Sabal palmetto* (Loyd et al. 2018). Differences in the capability of wood decay have been identified between isolates belonging to the same *Ganoderma* species (Adaskaveg et al. 1990).

In this study the suitability of different lignocellulosic waste originating from the sawmill industry on the growth of *G. lucidum* are explored. This study is the first to demonstrate the cultivation *in vitro* of *G. lucidum* originating from the northern Europe using wood residues as substrates. The effects of the wood species and agar media on the mycelial growth and morphology of different *G. lucidum* strains originating from different tree host species are studied.

Table 1 Fungal strains used in this study

Strain	Species	GenBank acc. no.	Province	Host species	Isolation date	Isolated by
MUS192	*G. lucidum*	MT334582	Uusimaa	*P. abies*	07/2016	Pyry Veteli
MUS**6**	*G. lucidum*	MT334583	Uusimaa	*P. abies*	07/2016	Pyry Veteli
MUS75	*G. lucidum*	MT334584	Uusimaa	*P. abies*	07/2016	Pyry Veteli
MUS9	*G. lucidum*	MT334585	Uusimaa	*P. abies*	07/2016	Pyry Veteli
MUS12	*G. lucidum*	MT334586	Satakunta	*P. abies*	10/2016	Pyry Veteli
MUS19	*G. lucidum*	MT334587	Uusimaa	*Betula pubescens*	03/2017	Pyry Veteli

Materials and methods

Fungal strains

Fungal strains were collected from wild fruiting bodies of *G. lucidum* growing on wooden stumps located in the Satakunta and Uusimaa provinces, in southern Finland. The host species was *Picea abies* for five strains and *Betula pubescens* for one strain. Six dikaryotic strains were isolated from the basidiomata and named MUS192, MUS6, MUS75, MUS9, MUS12 and MUS19 **(Table 1)**. The strains were maintained at 25 ˚C on 2% potato dextrose agar (PDA) and subcultured every three weeks. The identification of the strains was carried out by applying DNA-based identification methods based on the internal transcribed spacer (ITS) gene region. PrepMan Ultra Sample was used for DNA extraction following manufacturer's reagent protocol (Applied Biosystems, Fosters City, CA, USA). The ITS region was amplified by a polymerase chain reaction (PCR) using ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') forward primer (Gardes & Bruns 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') reverse primer (White et al. 1990). The thermal cycling protocol consisted of an initial denaturation cycle (95˚C for 3 minutes) followed by 35 cycles of denaturation, annealing and extension steps (95˚C for 30 seconds, 52˚C for 30 seconds and 72˚C for 1 minute, respectively) and a final extension step (72˚C for 10 minutes). The ITS sequences were deposited in the GenBank database **(Table 1)**. The strains are maintained in the Fungal Culture Collection of the Natural Resources Institute Finland (Luke).

Culture media

The culture media in 8.5 cm diameter Petri dishes were prepared using 2% malt extract agar [MEA: 20g malt extract/l (VWR International, LLC, USA), 15g agar bacteriological/l (VWR Chemicals, LLC, USA)], 2% potato dextrose agar [PDA: 20g potato dextrose agar/l (MP Biomedicals, LLC, France)] and water agar (WA: 15g agar bacteriological/l). Culture media with wood residues were prepared adding 20g/l of sawdust (dry weight) to the described agar media (MEA, PDA and WA). Wood chips and sawdust were obtained from the sawmill industry from North Karelia, in Finland. The wood chips were ground to a particle size of approximately 0.8 mm. After grinding, the particle size of the wood chips was homoge-

nous and similar to the sawdust. The wood residues were not further treated before their utilisation as substrate media. Sawdust from six different tree species was used: *Betula* spp. (*Betula pendula* with small fraction of *B. pubescens* sawdust), *Populus tremula*, *Larix* sp., *Pinus sylvestris*, *Alnus incana* and *Picea abies*. The *P. abies* sawdust consisted of a mixture of wood and bark. The media without wood substrate added served as control.

Mycelial growth

Five replicates were prepared for each combination of wood sawdust, agar media and strains. An agar plug (5 mm in diameter) from the actively growing margins of the 7-day old colonies was inoculated on the culture media and placed in the dark at 25°C. Mycelial growth was evaluated by measuring the mean radial growth on the media surface. The growth was measured by the colony diameters (two replicate measurements in two perpendicular directions from each plate) and the mean of the measurements was used in the analysis. The colony growth was measured every two days after the inoculation. After 18 days since the inoculation, measurements were taken every four days due to slow mycelial growth observed. The growth was followed for six weeks or until the agar plates were fully grown. The results were calculated in cm (± standard deviation) at each time point.

Morphology of the colony

Observations on the morphological characteristics of the colony were taken when the agar plates were fully grown or when mycelial growth was not observed anymore. The criterion used to examine the morphological characteristics was based on the methodology described by Sobal et al. (2007) with a modification including additional terms to describe the colony (Engelkirk & Duben-Engelkirk 2008). The morphological characteristics were analysed according to the following observed characteristics: complete colonisation, colony growth, exudates, mycelial density, aerial hyphae, colour, texture and topography **(Table 2)**.

Table 2 Morphological features used for the characterization of *G. lucidum* mycelia

Morphological feature	Observations
Complete colonization	Yes; No
Colony growth	Regular; Irregular
Exudates	Present; No present
Mycelial density	High; Medium; Low
Aerial Hyphae	Abundant; Regular; Scarce
Colour	White; Yellow; Red; Brown
Texture	Velvety; Cottony; Granular; Glabrous
Topography	Flat; Folded; Rugose; Umbonate; Crateriform; Verrucose; Cerebriform

Statistical analysis

Due to the longitudinal data structure (i.e., repeatedly measured plates), mixed-effect modelling was applied (e.g. Snijders & Bosker 1999). As a result, observations on the mean colony diameters on different measurement occasions were correlated; this can be taken into account by random plate effects in a variance component model (Searle et al. 1992). In the analyses, a measurement occasion was treated as a factor because it provided relevant information on the behaviour of the fungus at different growth stages.

The effects of the strain of *G. lucidum*, agar media, wood substrate and time since inoculation on the mycelial growth, were evaluated by the following mixed-effect model (Eqn.1): $d_{ijklt} = S_j + A_k + W_l + T_t + S_j * A_k + S_j * W_l + S_j * T_t + A_k * W_l + A_k * T_t + W_l * T_t + S_j * A_k * W_l + u_{ijkl} + e_{ijklt}$ where d = the mean colony diameter (cm), S = the strain of *G. lucidum* (j = 1, 2, ..., 6), A = agar media (k = 1, 2, 3), W = the wood substrate (l = 1, 2, ..., 7), T = the time after inoculation of the plate (t = 2, 4, ..., 42), u the random plates effect (five replicates per each combination of strain, agar and wood, i = 1, 2, ..., 5) with a mean of 0 and constant variance

Table 3 Type III test conducted on the mixed model (Eqn.1) to test the effect of the fixed factors strain, agar media, wood substrate and time since inoculation o n the radial growth of *G. lucidum*

Source	Numerator df	Denominator df	F	*p*-value
Intercept	1	508.2	13837.1	<0.001
Strain	5	508.8	16.9	<0.001
Agar	2	510.2	363.0	<0.001
Wood	6	510.2	20.4	<0.001
Time	20	691.5	1478.1	<0.001
Strain * Agar	10	471.6	11.7	<0.001
Strain * Wood	30	471.6	3.4	<0.001
Strain * Time	64	746.4	8.5	<0.001
Agar * Wood	12	469.5	13.1	<0.001
Agar * Time	40	586.2	39.5	<0.001
Wood * Time	120	594.6	9.0	<0.001
Strain * Agar * Wood	60	469.5	2.7	<0.001

and e = the random error term with a mean of 0 and heterogeneous variance over time. All two-level interaction terms were included in the model. To avoid overfitting, only the three-way interaction of the strain, agar and wood was considered in the model.

The model was fitted and estimated marginal means for all level combinations of the strains, agar media, wood substrates and time points were predicted using the model fitted by the MIXED procedure in IBM SPSS Statistics 25 (IBM SPSS Inc. 2017). Since the model involves random effects, the predicted means were computed by averaging the random effects over the subjects (i.e. the plates). Then for each level of time, all pairwise comparisons for the strain, agar media and wood substrate were performed to find out which strains, agar media and wood substrates had statistically significantly higher mean colony diameters at the time points of 10 and 18 days. Similarly, individual strains were compared on different wood substrates. Thus, the mean colony diameters presented in the results are the marginal means of the dependent variable predicted for the given factors (Strain, Agar, Wood, Time), adjusted for any other variables in the model (Eqn.1).

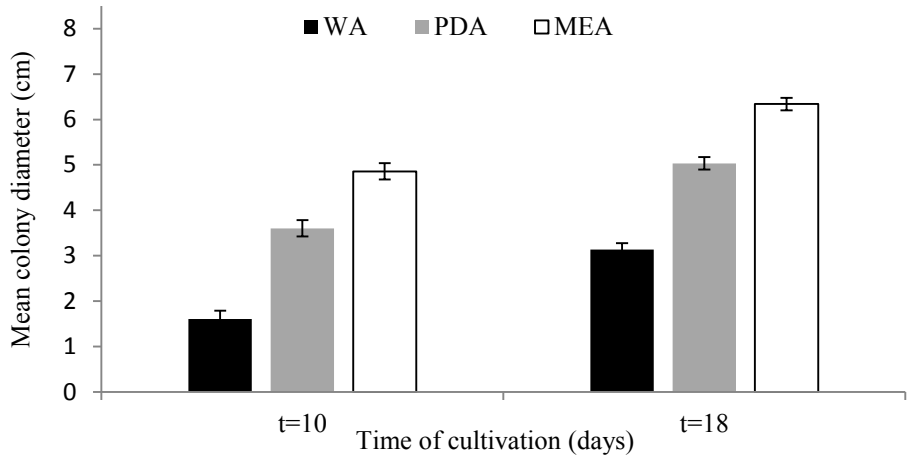

Fig 1. Effect of agar media on *Ganoderma lucidum* mycelial growth after 10 and 18 days of agar plate inoculation. All combinations of strains and sawdust amended media are included in the statistical analysis. All agar media are significantly different (p-value < 0.05) at each time point. Abbreviations: WA = Water agar, PDA = Potato dextrose agar, MEA = Malt extract agar.

Results

The mixed model analysis showed that all the main effects and their interactions had a significant effect (p-value < 0.001) on the variable d, mean colony diameter **(Table 3)**. As expected, the measurement occasion, i.e. the time since inoculation, had the strongest effect [F (20, 691.5) = 1478.1] on the mycelial growth. The estimated variance for the random between plate effects was 0.8049 (SD 0.90 cm). The heterogeneity of the error variance over time was notable. In general, the magnitude of random error terms was at its highest at the beginning and end of the experiment, and at its lowest between the time points 12 and 22 days after inoculation (variance < 0.5). In the following, the results on the main effects: strain [F (5, 508.8) = 16.9], agar media [F (2, 510.2) = 363.0] and wood substrate [F (6, 510.2) = 20.4] are presented in more detail.

Effect of the agar media composition

Ganoderma lucidum mycelial growth was observed in water agar (WA), potato dextrose agar (PDA) and malt extract agar (MEA). To determine the effect of the agar media composition all strains and sawdust amended media were included in the statistical analysis. Observations of the mean colony diameters in WA, PDA and MEA 10 and 18 days after inoculation are shown in **Figure 1**. The maximum mycelial growth, 8.5 cm, was achieved in most of the replicate cultures growing on PDA and MEA. Hence, as expected, the addition of potato dextrose and malt extract to the agar media enhanced the growth of *G. lucidum*. The mycelial growth (mean colony diameter, cm) was significantly different in each one of the agar media tested samples (p-value < 0.001) after 10 and 18 days of inoculation. MEA was found the most suitable composition for *G. lucidum* growth **(Figure 1)**. PDA was found the second most suitable agar media composition. Not surprisingly, the slowest mycelial growth was observed in WA.

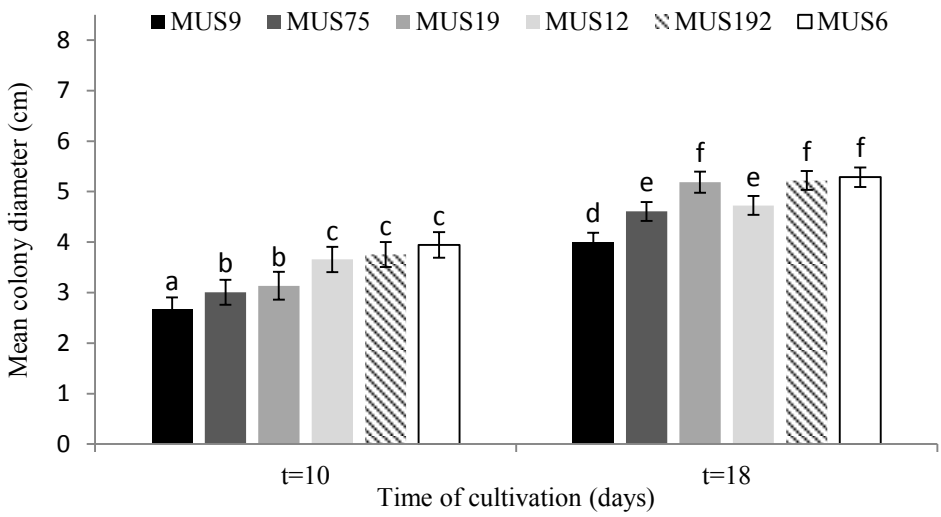

Fig. 2 Effect of strain on *Ganoderma lucidum* mycelial growth after 10 and 18 days on agar plates. All combinations of agar composition and sawdust amended media are included in the statistical analysis. Significant differences (p-value < 0.05) between strains at the same time point are represented with different letters.

Regarding the colony characteristics, large differences in the mycelium growth were observed between the media containing WA and PDA or MEA. As expected, the mycelia had a very low density and the hyphae were thin and weak in WA. The growth distribution of the colony was irregular and resembled the root system of the trees. In most of the cases, the observation of the hyphae present in WA was rather difficult to the naked eye.

In the cases of the PDA and MEA media, the texture and the topography of the colony were variable. Aerial hyphae were often observed in the PDA media and less frequently in the MEA media. The colour of the hyphae varied from orange to dark red, especially darker in the colonies growing in the MEA media. Exudates were observed in the mycelia growing in both PDA and MEA but were absent in WA.

Effect of the strain

Six strains of *G. lucidum* were tested to determinate the strain-specific differences in mycelial growth. For this, all sawdust amended media and agar composition were considered in the statistical analysis and the mean growth results are shown after 10 and 18 days of inoculation **(Figure 2)**. The strains presenting higher mycelial growth were MUS6 and MUS192 after 10 days (not statistically different from MUS12) and 18 days (not statistically different from MUS19) of inoculation. MUS9 was the strain that had the significantly lowest mycelial growth. MUS75 presented the second lowest mycelial growth after 10 days (not statistically different from MUS19) and 18 days (not statistically different from MUS12) of inoculation. All strains were able to achieve the maximum mycelial growth, 8.5 cm, after 24 days of the inoculation of the agar plates. However, not all the replicates were able to colonise the entire plate (8.5 cm diameter). MUS192 and MUS19 had higher numbers of replicates that were able to fully colonise the plates by the end of the experiment. On the contrary, MUS75 and MUS12 had the lowest number of replicates able to achieve the maximum mycelial growth.

Regarding the colony characteristics, differences between the strains were observed. The mycelial

density was higher for the MUS6 and MUS19 strains; the density was medium for MUS192 and MUS12 and low for the MUS75 and MUS9 strains. The texture of the colony was variable in the cases of MUS6 and MUS19. The other strains presented a predominantly glabrous texture and a less frequently granular texture. All the strains presented highly variable topography. Aerial hyphae were not frequently observed in most of the strains except for MUS12 and MUS19. The hyphae presented a white colour and tonalities varying from orange to dark red, especially for the MUS75 strain. All the strains tested had a few replicates presenting exudates on the surface of the colony.

The growth of the colony was irregular in half of the replicates for all the strains. Irregular growth was defined when the hyphae grew submerged from the point of inoculation and therefore, the mycelium started colonising the plate from the extreme of the plates inwards. The area colonised from the extreme of the plate inwards presented, in general, a granular texture and aerial hyphae with low density.

Effect of the wood substrate

The effect of wood substrate on the mycelial growth of *G. lucidum* was determined including all observations of strains and agar composition in the analysis **(Figure 3)**. *Betula* spp. and *P. tremula* sawdust amended media significantly presented the highest mycelial growth during the entire cultivation period. After 10 days of inoculation, other sawdust amended media including *P. sylvestris*, *Larix* sp., *A. incana* and *P. abies* increased the mycelial growth significantly compared to the media without sawdust. Nevertheless, after 18 days, the mycelial growth on agar without sawdust was found to be significantly higher than on agar media containing *P. sylvestris*, *Larix sp.*, *A. incana* and *P. abies* sawdust.

The colonies growing on the media containing *P. tremula*, *A. incana* sawdust and without sawdust

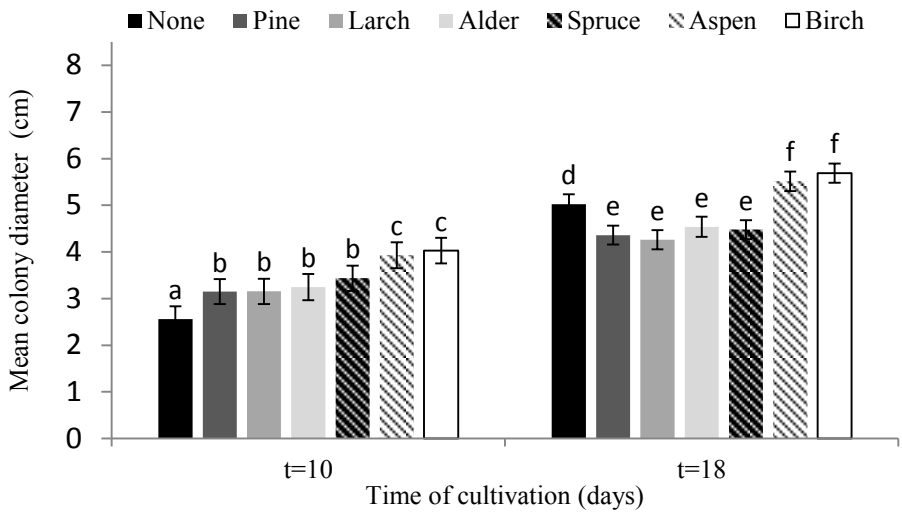

Fig. 3 Effect of wood substrates on *Ganoderma lucidum* mycelial growth after 10 and 18 days on agar plates. All combination of strains and agar composition are included in the statistical analysis. Significant differences (p-value < 0.05) between wood species at the same time point are represented with different letters. Abbreviations: None = No sawdust addition, Pine = *P. sylvestris*, Larch = *Larix* sp., Alder = *A. incana*, Spruce = *P. abies*, Aspen = *P tremula*, Birch = *Betula* spp.

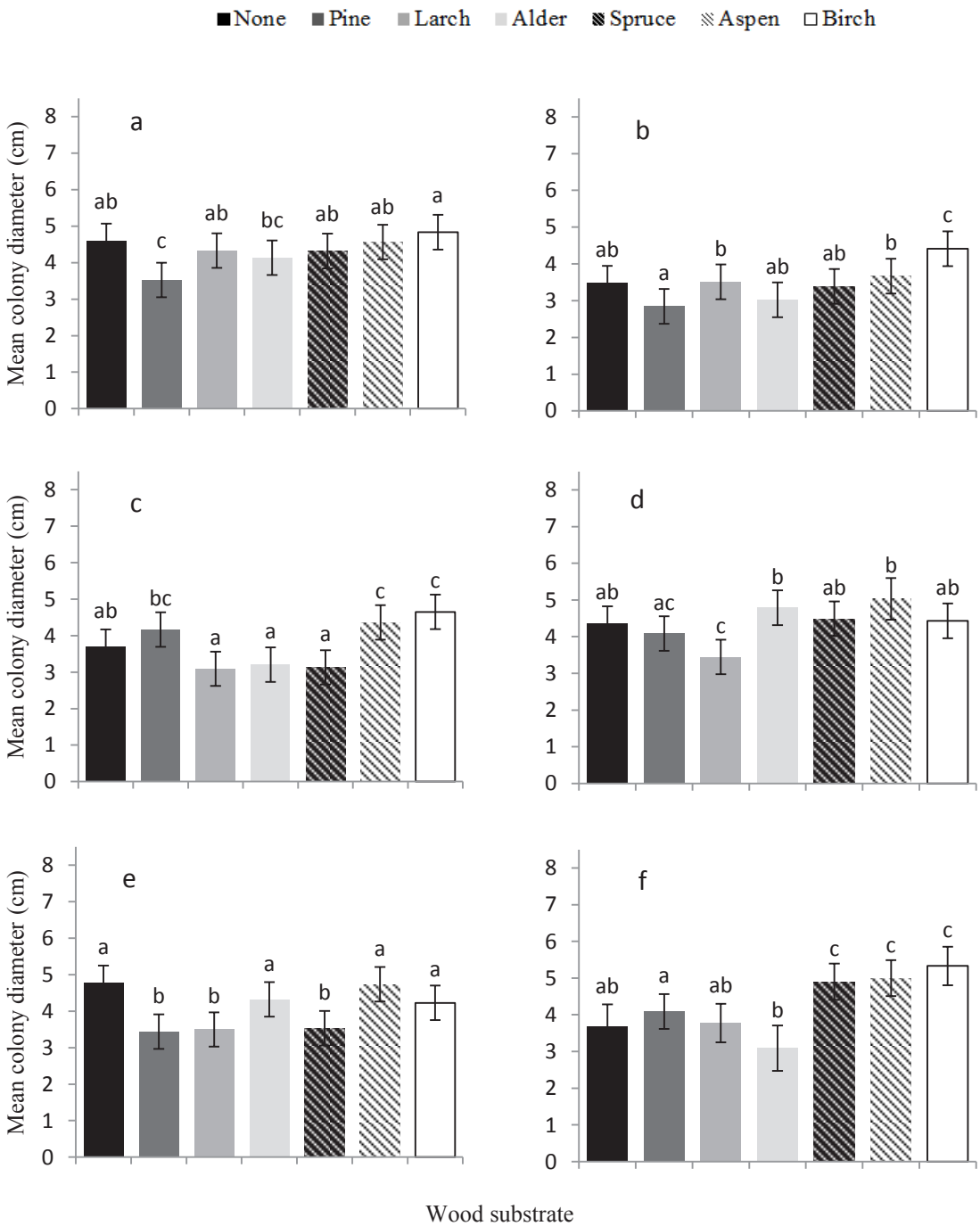

Fig. 4 Effect of wood substrates on the mycelial growth of different strains of *Ganoderma lucidum* after 6 weeks on agar plates. All agar composition are included in the statistical analysis. 4a Strain MUS192, 4b Strain MUS9, 4c Strain MUS75, 4d Strain MUS6, 4e Strain MUS12, 4f Strain MUS19. Significant differences (p-value < 0.05) between wood substrates are represented with different letters. Abbreviations: None = No sawdust addition, Pine = *P. sylvestris*, Larch = *Larix* sp., Alder = *A. incana*, Spruce = *P. abies*, Aspen = *P tremula*, Birch = Betula spp.

addition had the most replicates able to colonise the entire plate. On the other hand, the media containing *P. sylvestris* sawdust had the lowest number of replicates able to fully colonise the media. The mycelial density was higher in the media containing *Betula* spp. and *A. incana*, and lower in the media with *P. sylvestris* and without the addition of sawdust. The texture of the colony was glabrous and granular for most of the media except for the one containing *Betula* spp. sawdust, for which the texture was strongly variable. Regarding the topography, the media that had wood sawdust were variable in their shape. In contrast, the media without wood sawdust presented a predominately flat topography. The presence of aerial hyphae was common for the media with *Betula* spp. and *Larix* sp. sawdust. The lack of aerial hyphae was frequently observed for the media containing *P. sylvestris* and the media without the addition of wood sawdust. The colour of the hyphae was white, especially in the cases where *P. abies* and *P. tremula* were present. High numbers of replicates of the media containing *Betula* spp. presented orange to red coloured hyphae. Despite the fact that exudates were not found in most of the replicates, all the media tested had from one to eight replicates that presented fluid secretion. The growth of the colony was irregular for most of the replicates, especially for the media containing *Larix* sp., *P. abies*, and *A. incana*. In contrast, the media without wood sawdust presented regular growth.

Effect of the wood substrate on the strains

Betula spp. and *P. tremula* sawdust improved the mycelial growth more than other wood substrates included in the analysis of all strains and agar compositions **(Figure 3)**. In the model for the mean colonial diameter, the interaction between the strain and wood substrate was also statistically significant **(Table 1)**. **Figure 4** illustrates how the performance of specific strains varied on different wood substrates six weeks after inoculation. All agar compositions are included in the statistical analysis. The mean colony diameters represented in Figure 4 include all the measurements taken since the plate inoculation until 6 weeks after inoculation.

The most favourable media for the growth of the MUS192 strain was the one containing *Betula* spp. sawdust (not statistically different from *Larix* sp., *P. abies* and *P. tremula* sawdust; **Figure 4a)**. The media with *P. sylvestris* sawdust (not statistically different from *A. incana* sawdust) had the lowest mean mycelial growth for MUS192. Similarly to the MUS192 strain, the mycelial growth of the MUS9, MUS12 and MUS19 strains on *Betula* spp. sawdust were significantly higher compared to *P. sylvestris* sawdust. For instance, MUS9 had the significantly highest affinity with *Betula* spp. sawdust in comparison to the other wood species or control media **(Figure 4b)**.

The mycelial growth on the media containing *Betula* spp. and *P. sylvestris* sawdust did not significantly differ from each other when the MUS6 and MUS75 strains were used. For instance, the mycelial growth on *P. sylvestris* sawdust was among the highest for the MUS75 strain (not statistically different from *Betula* spp. and *P. tremula*; **Figure 4c**).

Alnus incana sawdust had high affinity with the MUS6 strain (not statistically different from *Betula* spp, *P. tremula*, *P. abies* and *P. sylvestris*; **Figure 4d**). Similarly, *A. incana* sawdust was among the most favourable media for the MUS12 strain (not statistically different from *Betula* sp. and *P. tremula*; **Figure 4e**). In contrast to this, the MUS192, MUS175, MUS9 and MUS19 strains inoculated on *A. incana* sawdust media did not behave as positively as the MUS6 and MUS12 strains.

Picea abies sawdust had the highest affinity with the MUS19 strain (not statistically different from *Betula* spp. and *P. tremula*; **Figure 4f**).

Discussion

In this work, several strains of *G. lucidum* were inoculated on a wide range of agar media combined with sawdust from different lignocellulosic biomass sources and compared in terms of mycelial growth and colony characteristics. The lignocellulosic biomass consisted of untreated wood residues from the sawmill industry readily available for utilisation as a substrate media. Substrates containing *Betula* spp. and *P. tremula* sawdust were the most suitable for the mycelial growth of *G. lucidum*. Additionally, fungal strain-specific differences were observed. Moreover, the natural host-tree species did not explain the wood substrate preference of *G. lucidum in vitro*. The results revealed a significant variability in the

behaviour of the strains on agar and wood substrate combinations. Considering the media without wood substrate added, MEA was found the most suitable for growth of *G. lucidum* in comparison to WA or PDA. Consistent with our results, Bilay et al. (2000) tested a variety of agar media and found that MEA provided a maximal growth rate of 3.44 mm/day for *G. lucidum* in a plate agar test. We expected that MEA and PDA would be suitable for the growth of *G. lucidum* since both media are commonly used for cultivating numerous fungi. MEA and PDA have been previously reported by Gbolagade et al. (2006) and Gizaw (2015) to support mycelial growth of other fungal species such as *Lentinus subnudus* and *Pholiota nameko*, respectively. Fungal morphological differences between the media can be due to different pH contents in MEA and PDA since it interferes in the mycelial growth rate of *G. lucidum* (Bilay et al. 2000). The reason MEA enhances the growth of certain fungal species could be allocated to the high content of vitamins, minerals and amino acids present in malt extract (Alizadeh et al. 2013).

Based on the colony characteristics results in this study, the addition of malt extract, potato dextrose and certain wood sawdust to the agar media promoted the density of the colony of *G. lucidum*. The density of the colony has been identified as a factor associated with favourable conditions of the media for fungal nutrient extraction (Zervakis et al. 2001). As a consequence, the presence of lignocellulosic biomass in the agar media served as a suitable nutrient source for *G. lucidum*.

In our experiment, *G. lucidum* strains had different growth responses to the wood substrate in the agar media. We expected that the addition of wood sawdust to the agar media would positively affect the growth of *G. lucidum*. However, the mycelial growth was found to be lower in agar media containing softwood sawdust in comparison to the media without sawdust or including hardwood sawdust. This finding suggests that the use of side streams of *P. sylvestris*, *P. abies* and *Larix* sp. would not be most preferable substrates for the cultivation of *G. lucidum*. On the contrary, the hardwood species containing media significantly enhanced the mycelium growth of *G. lucidum* in comparison to the media without sawdust, especially the media containing *Betula* spp. and *P. tremula* sawdust. The addition of *A. incana* sawdust was not as favourable as the other hardwood species in this respect.

Hardwood species, such as oak or poplar, are commonly used for the cultivation of *G. lucidum* (Erkel 2009). In Nordic temperate and boreal forests, *G. lucidum* has been previously found in *Betula* spp. and *P. abies* (Kajava & Silver 2016). According to Loyd et al. (2018), the tree species specific performance of strains may be due to the affinity of the strains with the host from which they were originally isolated in nature. We expected to observe an affinity between the strains and the wood species in this experiment; however, we did not observe this correlation. For instance, the strain with highest mycelial growth observed in *P. abies* sawdust was MUS19, whose original host tree was *B. pubescens*. All the other strains were isolated from fruiting bodies growing on *P. abies* and performed better in media with hardwood sawdust.

The chemical composition and physiological features of the wood material play an important role in the success of *Ganoderma* spp. colonisation and degradation ability (Loyd et al. 2018). Baietto & Wilson (2010) carried out several wood decaying tests using nine tree species and found higher decay rates of *G. lucidum* when hardwood blocks were used. The same occurred with other *Ganoderma* spp. (Adaskaveg et al. 1990). Despite the fact that the mycelial growth rate does not reflect the wood decay ability of fungi, the differences observed in this study could be justified by the chemical and physiological characteristics of the wood used.

Differences in the fungal growth response to the wood species could be due to the extensive genetic variability between and within populations of strains (Urbanelli et al. 2003). For instance, Wymelenberg et al. (2011) found that gene expression patterns of white rot and brown rot fungi (*Phanerochaete chrysosporium* and *Postia placenta*, respectively) were significantly influenced by hardwood (*Populus grandidentata*) and softwood (*Pinus strobus*) substrates. Understanding the genetic information of *G. lucidum* strains is essential to select the most suitable for commercial production. Moreover, due to the current taxonomic confusion of *G. lucidum*, we emphasise the importance of clarifying the species boundaries in future studies.

The present work demonstrates the impor-

tance of strain and substrate media selection for *G. lucidum* cultivation. Wood residues from sawmill industries without previous treatment served as growth media for *G. lucidum*. Wood residues have primarily been used for energy production; the findings of this study open a new opportunity for the use of wood residues as a raw material for the cultivation of *G. lucidum*. Further research on fruiting body production, biomass conversion and degradation capacity of lignocellulosic material will be necessary to better understand the suitability of the wooden substrates for mushroom cultivation. The selection of the best performing combination of strains and wood species could be translated into shorter cultivation periods of *G. lucidum* and more efficient conversion of low value lignocellulosic biomass into high valuable crops.

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