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Selective breeding and taxonomy of laccate *Ganoderma* species originating from Finland

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Academic dissertation
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ABSTRACT

Ganoderma lucidum has been widely cultivated worldwide due to its bioactive compounds. Agricultural and forestry by-products have been used as growth substrate for the cultivation of *G. lucidum*. In Finland, there are high volumes of wood biomass by-products that could be used for the cultivation of this fungal species. A laccate *Ganoderma* species resembling *G. lucidum* occurs naturally in Finnish forests, but there is lack of information on the wild population. To cultivate Finnish strains of *G. lucidum*, it is essential to study the variability of the wild populations of the species and select the most appropriate for the industrial use. In this dissertation, the phenotypic variation, the cultivation practices, the *β*-glucan content, and the phylogeny of laccate *Ganoderma* originating from Finland have been explored. The growing conditions were adjusted, and the suitability of the strains, supplements, and substrates tested. Finnish strains of *G. lucidum* were successfully cultivated using wood by-products as substrate. A cold shock treatment of the colonized substrate was needed to trigger the formation of fruiting bodies. Substrates based on *Populus tremula* and *Betula* sp. sawdust and wood chips increased the probability of fruiting, fruiting body yield, and *β*-glucan content of *G. lucidum*. Substrate based on *Pinus sylvestris* wood was not suitable for mushroom production, but the fermentation by *G. lucidum* decreased the lignin content of pine wood, which could be a potential pretreatment for pulping industries. Phenotypic variability of the strains was noted based on mycelial growth, mycelium and fruiting body morphology, fruiting body yield, and their ability to produce *β*-glucan. Despite the phenotypic variation observed within the Finnish *G. lucidum* populations, the Finnish isolates presented nearly identical gene regions based on multiple gene regions and grouped together in the phylogenetic trees generated. The Finnish strains of laccate *Ganoderma* were confirmed to represent a single species according to the phylogenetic analysis. More specimens and molecular data are needed to clarify the classification of laccate *Ganoderma* from Finland.

Keywords: Circular economy, fermentation, Ganodermataceae, lignocellulosic substrate, mushroom cultivation, polysaccharides.

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Joensuu, 19th November 2024

Marta Cortina-Escribano

LIST OF ORIGINAL ARTICLES

This thesis is based on data presented in the following articles, referred to by the Roman Numerals I-IV.

I Cortina-Escribano M, Veteli P, Linnakoski R, Miina J, Vanhanen H (2020) Effect of wood residues on the growth of *Ganoderma lucidum*. Karstenia 58: 16–28. https://doi.org/10.29203/ka.2020.486

II Cortina-Escribano M, Pihlava JM, Miina J, Veteli P, Linnakoski R, Vanhanen H (2020) Effect of strain, wood substrate and cold treatment on the yield and *β*-glucan content of *Ganoderma lucidum* fruiting bodies. Molecules 25: 4732. https://doi.org/10.3390/molecules25204732

III Cortina-Escribano M, Barbero-López A, Kilpeläinen P, Vanhanen H, Haapala A (2024) Degradation of *Pinus sylvestris* and *Populus tremula* by laccate *Ganoderma* species. Holzforschung. https://doi.org/10.1515/hf-2024-0052

IV Cortina-Escribano M, Veteli P, Wingfield MJ, Wingfield BD, Coetzee MPA, Vanhanen H, Linnakoski R (2024) Phylogenetic analysis and morphological characteristics of laccate *Ganoderma* specimens in Finland. Mycologia: 1–17. https://doi.org/10.1080/00275514.2024.2381424

The present author was the principal author of all the articles, with the main responsibility for the experimental design and realisation, analysis, visualization and reporting of the results. The results were also partially analysed by the fourth author in Article I, the second and the third authors in Article II, and the second author in Article IV. The present author wrote the first draft for the articles and was the corresponding author during the submission to journals. The other co-authors participated in the experimental design and/or writing and editing of the articles.

TABLE OF CONTENT

List of abbreviations:

BI	Bayesian inference
DNA	Deoxyribonucleic acid
ITS	Internal transcribed spacer
MEA	Malt extract agar
ML	Maximum likelihood
MP	Maximum parsimony
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PSK	Polysaccharide krestin
PSP	Polysaccharide peptide
RAPD	Random amplified polymorphism DNA
RPB2	RNA polymerase II subunit
TEF1	Elongation factor 1-α
WA	Water agar
β-tub	Partial β-tubulin

INTRODUCTION

Background and motivation

The European Union is incentivising the swift towards a circular bio-based economy by putting into practice strategies and policies to accelerate the transition (European Commission 2012, 2021). Significant part of this activity is the commercialization of new bioproducts, substitution of non-renewable materials and chemicals with bio-based alternatives, and support for the associated research, development, and innovation. Several European countries have dedicated Bioeconomy strategies at national level and their implementation is funded by European and national instruments. In Finland, the initiative is being adapted by implementing the current Finnish Bioeconomy Strategy (2022), in which one of the target sectors have been the forestry industry and one of the main goals is the efficient use of the biomass resources. This strategy supports the research and establishment of new business models and value chains to transform forestry and agricultural by-products into higher value materials and chemical products. The strategy also promotes identifying new opportunities to increase the value added in the natural products sector, which includes mushrooms, berries, sap and other non-timber forest products.

The forestry sector in Finland produces a large amount of different side-stream fractions with currently quite limited valorisation potential. The further use of these by-products depends on their characteristics, and can be divided in high, medium, or low-value side-streams (Hassan et al. 2018). Most of the forestry by-products are considered of medium-value and consist of bark, sawdust, wood chips, treetops, and stumps, which are mostly used for energy production (i.e. fuel, heat and pellet production) and, in a minor scale, as raw material for other processes such as pulp, particleboards or fibreboards (Hassan et al. 2018). Different future scenarios to utilize further forestry by-products in Finland were presented by Kunttu et al. (2020), and those include: the current use for pulp and bioenergy production, the production of long-lifetime product (i.e. wood composites, particleboards, and fibreboards), and the development and integration of new bioproducts (i.e. Zhao et al. 2021).

The Fungi kingdom may have an emerging role in achieving some of the Finnish bioeconomy targets and in the development of new bioproducts by allowing the transformation of lignocellulosic low and medium-value side-streams into higher-value products. Saprotrophic fungi can decompose forestry and agricultural by-products to produce specialty and edible mushrooms as well as fibres, polysaccharides, and bioactive compounds (Atila et al. 2018; Rashad et al. 2019; Kurd-Anjaraki et al. 2022; Castorina et al. 2023). Several Asian countries have successfully implemented mushroom cultivation techniques and fungal bioprocesses to up-cycle by-products of agriculture and forestry industries (Li and Xu 2022). However, the forestry and agricultural industries in Northern Europe are not familiar with fungal cultivation practices. Moreover, the mushroom industry in Europe is limited to only few commercial cultivated species (Singh et al. 2020).

Ganoderma lucidum (Curtis) P. Karst. is a popular commercial species that has been widely cultivated and consumed specially in East-Asian countries due to its bioactivity. The bioactivity of *G. lucidum* is associated to the content of polysaccharides, including *β*-glucan, and secondary metabolites. *Ganoderma lucidum* derived products have been industrially used

to manufacture food, beverages, feed, dietary supplements, pharmaceuticals, nutraceuticals, cosmetics, and personal care products (Hapuarachchi et al. 2018). In recent years, *Ganoderma lucidum* have been also used in the textile, packaging, and construction industry (Vandelook et al. 2021). The global market value of *Ganoderma* products was valued at 4.3 billion USD in 2023 and it is expected to increase at a compound annual growth rate of 9% (the Business Research Company 2024).

Ganoderma lucidum is naturally distributed in Finland (Niemelä and Kotiranta 1986), but its occurrence is rare. Considering the extensive availability of forestry and agricultural side-streams in Northern Europe, the cultivation of *G. lucidum* could be a potential solution for the creation of new bioproducts and value chains (i.e. cascade processing). In Northern Europe, the mushroom cultivation industry is limited to the cultivation of few fungal species, of which *G. lucidum* is not among them. Therefore, there are not previous reports on the cultivation of *G. lucidum* in Northern Europe using local fungal strains and resources. Also, there is limited background information on the bioactivity, cultivation potential, and taxonomy of the Finnish populations of *G. lucidum*. To evaluate the cultivation potential of *G. lucidum* in Finland, it is crucial to understand the variability of local laccate *Ganoderma* strains, their bioactivity potential using *β*-glucan as a marker, their taxonomic relation with the European originally described species, and to optimize the cultivation technique using forestry and agricultural by-products. The present work describes the first research on strain comparison, selective breeding, *β*-glucan content, and phylogenetic analysis of *G. lucidum* originating from Finland. This is the first report on the successful cultivation of *G. lucidum* in the Nordic countries.

Mushroom cultivation industry

Fungi play an important role in ecosystems as well as in the global economy, supporting the transition towards a biobased circular economy (Niego et al. 2023). Fungi are a rich source of proteins, amino acids, carbohydrates, vitamins, fats, minerals, and bioactive compounds, which have been used as food, but also in several industrial applications such as cosmetics, cosmeceuticals, and nutraceuticals (Niego et al. 2023). The global mushroom cultivation market was valued at around 28.5 billion USD in 2022, and the compound annual growth was estimated at 4.6% (Market Research Guru 2024). The mushroom cultivation industry has the major representation in many Asian countries. According to FAOSTAT (2024) the leading mushroom producing country in the world is China followed by Japan and the Republic of Korea, which produced approximately 94%, 1% and 0.05% from the global mushroom production share in 2022. During the same year, the production of mushrooms in the whole of Europe accounted for 2.8% of the total global share. Here most of the production focuses on Poland, Netherlands, Spain, and France (FAOSTAT 2024). Thought FAOSTAT data does not cover the entire mushroom industry, it gives a broad estimation of the world mushroom production (Singh et al. 2020).

Despite the high number of edible fungal species available, only few are actively and extensively cultivated and commercialized (Zhang et al. 2014). The fungal species globally cultivated are limited to few genera, such as *Agaricus*, *Auricularia*, *Flammulina*, *Lentinula* and *Pleurotus* (Singh et al. 2021). The most cultivated species in China are *Lentinula edodes*, *Auricularia auricula*, *Pleurotus ostreatus*, *Flammulina velutipes*, *Agaricus bisporus*, *Pleurotus eyngii*, and *Auricularia polytricha* (Singh et al. 2020). Li and Xu (2022) identified several challenges of the mushroom industry to commercialize and expand the mushroom

market in China, some of those are: the high competition in the industry, the proximity of raw material or feedstock to the mushroom industries (resource availability), the energy consumption and the incorporation of green energy, the need for innovation in terms of new products and technologies, and popular awareness of nutritional and functional properties of the commercial fungal species.

In Europe, the mushroom industry has been mostly focused on the cultivation of *Agaricus bisporus*, *Pleurotus* spp. (*Pleurotus ostreatus* and *Pleurotus eryngii*), and *Lentinula edodes*; less than 4% of the mushroom production corresponds with other fungal species (Singh et al. 2020). Higher-valued fungal species could be cultivated by using existing side-streams of agro- and forestry industries as growth substrate, such as the case of *Ganoderma lucidum*. *Ganoderma* derived products are successfully marketed in Asia, Europe, and North America with an estimated global market of 4.3 billion USD; however, its production is mostly concentrated in the few Asian countries (Niego et al. 2023). In nature, *G. lucidum* is rarely found in Finnish forests. But the large availability of wood by-products produced by the forestry industry in Finland could be used for the commercial cultivation of this naturally occurring species.

Objectives of the study

Aim of the thesis

The general aim of this thesis was to investigate the substrate suitability, the cultivation conditions, the content of *β*-glucan, and the taxonomy of *Ganoderma lucidum* originating natively from Finland to enhance its potential applications in the functional food industry. Therefore, the objectives of the thesis are divided into following aspects (Figure 1):

1) to identify the environmental and substrate factors influencing mycelial growth and mycelial characteristics of *Ganoderma lucidum* originating from Finland (Articles I and III);
2) to determine the optimal wood substrate, strain, and cultivation conditions for fruiting body development and *β*-glucan production (Articles I and II) and;
3) to clarify the taxonomy of the laccate *Ganoderma* from Finland, facilitating accurate classification and a better understanding of species-specific characteristics (Article IV).

Research hypotheses

1) Wood by-products from Finnish industries are suitable for the cultivation of *Ganoderma lucidum*.
2) The optimal cultivation substrate is related to the tree host species of the fungal strain.
3) There is a performance variation between strains of *Ganoderma lucidum* originating from Finland.
4) *β*-glucan production can be optimized by wood substrate and fungal strain selection.
5) The laccate *Ganoderma* species naturally occurring in Finland corresponds with *Ganoderma lucidum*.

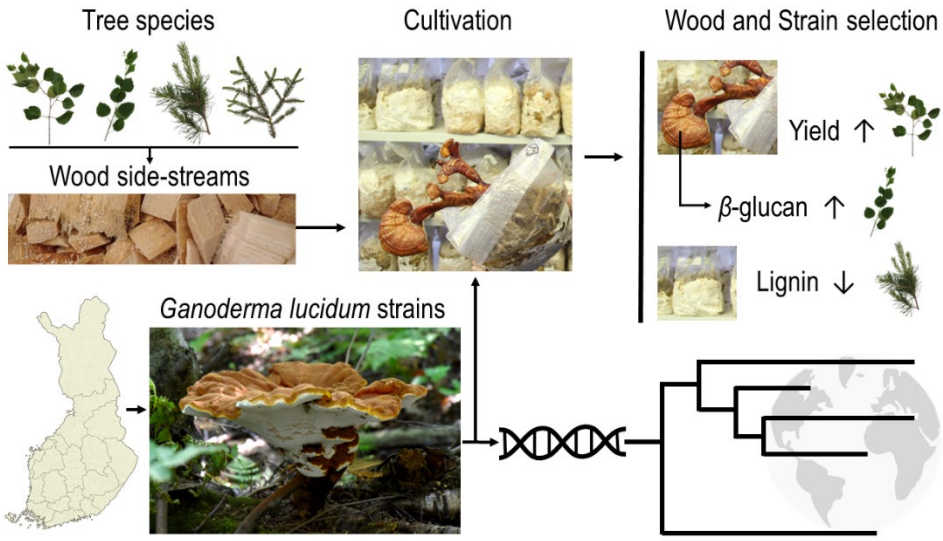

Figure 1 Objectives of the dissertation. Pictures by Henri Vanhanen and Pyry Veteli. Graphics by Henri Vanhanen, Alejandro Alamán and Marta Cortina-Escribano.

CULTIVATION AND TAXONOMY OF *GANODERMA LUCIDUM*

Life cycle of *Ganoderma lucidum*

The *Ganoderma* genus includes wood-decaying polyporoid species that are pathogenic causing root and stem rot in trees or saprotrophic causing white-rot of wood from dead trees (Kües et al. 2015). In Finland, *G. lucidum* is saprotrophic and it is found growing on tree stumps of both broadleaf and conifer species (Niemelä and Kotiranta 1986). The distribution of *G. lucidum* in Finland is mostly restricted to the Southern boreal region (Niemelä and Kotiranta 1986). The species has been reported to occur also in North Karelia and North Ostrobothnia (Pyry et al. 2019), and an occurrence has been reported in Pello, above the Arctic circle (the Finnish Biodiversity Information Facility).

As for most basidiomycete species, the life cycle of *G. lucidum* is dominated by the mycelium phase. The mycelium is the vegetative structure of fungi and consists of a hyphal network that is developed after the germination of a spore. *Ganoderma* species are heterothallic basidiomycetes, which means that mating of compatible monokaryons is required to produce fruiting bodies. Tetrapolar species, such as the case of *Ganoderma* species, have two different regions in the genome that contain genes encoding transcription factors and pheromone receptors (Kothe 1996); these are responsible of the mating of monokaryotic mycelia and the formation of dikaryotic mycelium, leading to the formation of fruiting bodies (mushrooms).

The development processes involved in the production of fruiting bodies have been thoroughly studied using *Coprinus cinereus* (Kües 2000), which is the model organism representative for basidiomycetes. The production of fruiting bodies starts with the formation

Figure 2 Primordia (right) and mature fruiting body (left) of *Ganoderma lucidum* occurring in wood stumps of *Betula pubescens* in North Karelia, Finland. Pictures by Henri Vanhanen.

of hyphal knots, which consist of highly branched dikaryotic mycelium that leads to the formation of multicellular structures called sclerotia. When exposed to light, the next development phase is triggered by the formation of primordia (Kües 2000). The development of primordia is followed by stipe and basidiocarp formation (Figure 2), where the basidiospores are formed and released.

Bioactive compounds from *Ganoderma lucidum*

Bioactivity can be defined as the determined effect of a substance on an organism or a living tissue (Williams 2022). Species belonging to the *Ganoderma lucidum* complex produce primary and secondary metabolites in their mycelium (Hirotani et al. 1986; Sun et al. 2021a; Sułkowska-Ziaja et al. 2022), fruiting bodies (Kozarski et al. 2012; Ćilerdžić et al. 2014; Li et al. 2018), and spores (Zhu et al. 2018; Salvatore et al. 2020; Sun et al. 2021b), which define the bioactivity of the fungi (Ahmad 2018).

The bioactive compounds of *G. lucidum* have shown antifungal (Wang and Ng 2006; Andrejč et al. 2022; Cör et al. 2022); antiarthritic (Cao et al. 2020), antibacterial (Ćilerdžić et al. 2014; Andrejč et al. 2022; Cör et al. 2022), anti-diabetic (Ryu et al. 2021), anti-inflammatory (Wu et al. 2019; Ryu et al. 2021; Cör et al. 2022), antimicrobial (Ćilerdžić et al. 2014; Cör et al. 2022), antioxidant (Kozarski et al. 2012; Ćilerdžić et al. 2014; Ryu et al. 2021; Andrejč et al. 2022; Cör et al. 2022), antiviral (Linnakoski et al. 2018; Andrejč et al. 2022; Cör et al. 2022) and antitumor (Ferreira et al. 2015; Li et al. 2018) effects. For instance, ethanol extracts of fruiting bodies (Ćilerdžić et al. 2014) and chitosan extracted from spore powder (Zhu et al. 2018) of *G. lucidum* have presented antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

The bioactive compounds of *G. lucidum* primarily include polysaccharides and secondary metabolites (Table 1), which are mainly responsible of its nutritional and pharmaceutical properties, driving the commercial applications of the species.

Secondary metabolites

Low molecular weight compounds produced by the fungi that are not essential for the fungal growth but are involved in the interaction of the individual with other organisms and the environment, as well as in the defence mechanisms of the organism (Macheleidt et al. 2016). In *G. lucidum*, the most studied group of secondary metabolites in term of bioactivity are terpenoids (Ahmad 2018).

Polysaccharides

High molecular weight polymerized compounds with physiological properties present in the fungal cell wall. Polysaccharides have a wide range of functions in the fungal cell wall such as protecting the cell against the environment and other organisms. Moreover, polysaccharides are essential components of the structure, strength, and shape of the fungal cell (Bowman and Free 2006). The bioactivity of *G. lucidum* have been mainly attributed to the polysaccharides extracted from the mycelium, fruiting bodies, and spores, and terpenoids of the fungi (Nie et al. 2013).

***β*-glucan**

Structural polysaccharide that has an influence on the rigidity of the fungal cell and serves as a carbohydrate reservoir (Zhu et al. 2016). *β*-glucans isolated from fungi have received special attention due to their bioactive properties such as immunomodulatory, anti-inflammatory, antioxidant, antitumor and immunomodulating properties (Rop et al. 2009; Khan et al. 2018; Zhang et al. 2023). Due to its bioactivity, fungal *β*-glucans are used in the food, medical, cosmetic, and feed industry (Du et al. 2014; Zhu et al. 2016; Zhang et al. 2023). The increasing demand of fungal derived *β*-glucans has encouraged the research on the optimization of cultivation techniques (Reverberi et al. 2004; Atoji-Henrique et al. 2017; Kiss et al. 2021; Article II) and extraction methods (Hwang et al. 2018; Vaithanomsat et al. 2022; Zheng et al. 2024) to increase the yield of *β*-glucan in fungi. For instance, Atoji-Henrique et al. (2017) analyzed the *β*-glucan content of different substrate media after the fermentation of *G. lucidum*. They obtained up to 180.3 mg/g of *β*-glucan in fermented soybean hulls.

Table 1 Bioactive compounds from *Ganoderma lucidum*

Group	Compound	Reference(s)
Polysaccharide	*β*-glucans, α-glucans, α-galactose, α-mannans, chitin, PSK, PSP	Nie et al. 2013
Terpenoid	Ganoderiol, lucidenic acid, lucialdehyde, ganoderic acids, ganolucidinic acid, ganodermantriol, lucidadiol	Ahmad 2018; Ahmad et al. 2022
Protein	Ganodermin	Wang and Ng 2006
Fatty acid	Palmitic acid, linoleic acid, oleic acid, stearic acid, tricosanoic acid, lauric acid, tetracosanoic acid	Lv et al. 2012; Tokul-Olmez et al. 2018
Mineral	Ca, K, P, Na, C, Fe, Zn, Mg	Shamaki et al. 2012

Health claims of *β*-glucan derived from oat and barley have been approved by the European Food Safety Authority (EFSA), as its consumption is associated to maintain blood cholesterol levels (EFSA 2009). Also, the US Food and Drug Administration (FDA) have approved a health claim on the relation of *β*-glucan intake and lowering the risk of coronary heart disease (FDA 2018). The recognition of *β*-glucan derived from oat and barley by the EFSA and FDA makes this bioactive compound interesting from the commercial perspective. Despite of the structural differences between cereal-derived and fungal-derived *β*-glucans (Hu et al. 2022), the approval of cereal-derived *β*-glucan as a health claim is the main motivation to use it as a marker of bioactivity for *G. lucidum* in the present work.

Factors affecting the production of polysaccharides

The composition and the content of polysaccharides and secondary metabolites vary between fungal species and within strains belonging to the same species. Some of the factors affecting the production of *β*-glucan and the composition of polysaccharides are the phenotypic variation within the species, the growth stage, the fermentation conditions, and the environmental factors (Hapuarachchi et al. 2019; Zhou et al. 2018; Zapata et al. 2009; Atoji-Henrique et al. 2017). These factors and conditions are considered and optimized by cultivators to the best of their knowledge to obtain a determined bioactivity and to enhance the production of certain bioactive compound or group of compounds.

Ganoderma species present a high **phenotypic plasticity**, which consist of the development of different morphological features from a determined genotype (Hapuarachchi et al. 2019). The phenotypic plasticity of a species is influenced by extrinsic (environmental and growing conditions) and intrinsic (genetic) factors (Hapuarachchi et al. 2019; Nishizawa et al. 2000). *G. lucidum* strains may present differences in their **metabolic profile** (Pawlik et al. 2015), and in their capacity to produce metabolites (Zhou et al. 2024) and polysaccharides (Article II). The phenotypic plasticity has been also observed between *G. lucidum* strains isolated from the same geographical region (Articles I and II) and presenting nearly identical gene regions (Article IV). In the mushroom industry, commercial strains are often used to decrease the variation of the expected fruiting body yield and/or bioactive compound profile (Oei 2016). However, the consecutive use of commercial strains for a single process may lead to the loss of genetic diversity (Pawlik et al. 2015). Some of the diagnostic features to evaluate the suitability of *G. lucidum* strains for commercial applications are the growing speed, the morphological characteristics, the enzymatic activity, the yield of fruiting bodies, *β*-glucan content, the yield of bioactive compounds, and the nutritional properties (Zhou et al. 2012).

Ganoderma lucidum experiences changes in the composition and production of polysaccharides and secondary metabolites during its life cycle and **developmental stages** (Chen et al. 2012; Zhou et al. 2018). In the initial growth stage of the fruiting body, the expression of genes involved in the biosynthesis of triterpenoids and ergosterols is high and decrease after the sporulation takes place (Cai et al. 2021), while the expression of genes involved in the synthesis of carbohydrates increased with the maturation of primordia into fruiting bodies (Chen et al. 2012). However, Zhou et al. (2018) reported a decrease on the content of soluble polysaccharides from 2.39% to 1.46% from primordia to mature fruiting body, respectively. Moreover, Zhou et al. (2018) reported different content of polysaccharides in the cap, the stipe, and the base of the fruiting body regions of *G. lucidum*.

The **fermentation conditions** and the **environmental factors** also contribute to the changes in the composition and production of *G. lucidum* polysaccharides. For instance, the

light exposure at different wavelengths had an effect in the production of mycelial biomass of *G. lucidum* under submerged conditions (Zapata et al. 2009), and in the shape (Sudheer et al. 2018b) and yield of fruiting bodies in solid-state fermentation (Montoya et al. 2018). The selection of the fermentation medium has also an influence on the yield and concentration of fruiting body polysaccharides of *G. lucidum* (Atoji-Henrique et al. 2017; Article II). Additionally, the exposure of *G. lucidum* to **external substances**, has been used to enhance the content of bioactive compounds. For instance, Hu et al. (2017) increased 85.96% and 63.90% the production of ganoderic acids of *G. lucidum* in submerged fermentation by adding microcrystalline cellulose and D-galactose, respectively. Sudheer et al. (2016) optimized the production of ganoderic acids, polysaccharides, phenolics and flavonoids by exposing *G. lucidum* fruiting bodies to different ozone concentrations.

Cultivation of *Ganoderma lucidum*

Strain breeding

Fungal species have a wide phenotypic and genetic variation among and within their natural populations (i.e. Nishizawa et al. 2000; Gao et al. 2013). Mushroom farmers and biotechnological industries often use commercial strains to reduce the variation of their crop quality and yield (Oei 2016). Selecting the appropriate strain is a key step for the successful cultivation of fungi (Kothe 2001). Some of the features used to select fungal strains are the growth rate, the fruiting body yield, the morphology of the fruiting bodies, the production of polysaccharides and secondary metabolites, their resistance against contaminants and other organisms, and their pharmacological properties. For instance, Liu et al. (2017) cultivated *G. lucidum* in a solid substrate and reported a fruiting body yield ranging from 9.46 to 21.40 g/g (fresh fruiting bodies per dried substrate) depending on the strain used. Tang et al. (2023), developed mutant strains of *G. lucidum* to increase their resistant ability to other organism invasions and contaminants. Therefore, the selection of the strain influences the production time and efficiency in the biotechnological processes.

There are several methods to select and breed fungal strains aimed for biotechnological processes, such as selective breeding, mutation breeding, cross-breeding, cell fusion breeding, and genetic engineering breeding (Zhou et al. 2012; Salazar-Cerezo et al. 2023). The breeding methods differ on the initial and end strain type, the background information required of the strain, and the mechanism pathway to obtain a new strain with determined features (i.e. biochemical characteristics, morphological aspect, or enzymatic activity). A general and simplified scheme to select a breeding strategy is shown in the Figure 3.

Selective breeding. In the mushroom industry this refers to isolating pure strains from the fruiting body tissue or spore print from a specimen with certain desired features (Zhou et al. 2012). Therefore, the strain is selectively cultivated to express the particular trait. For instance, Xu et al. (2021), isolated several strains from wild fruiting bodies of *G. lucidum* occurring in Zheijang Province in China, and compared them with commercial strains. One of the isolated strains outperformed the commercial strains presenting higher polysaccharides, bioactive compounds, and spores; also, the time needed for fruiting was reduced by 6 days.

Cross-breeding. The mating of monokaryotic or haploid strains. Desired features from different parent strains are expected to express in the newly formed dikaryotic strain. The parent strains must be the same species or close-related species to be able to produce new

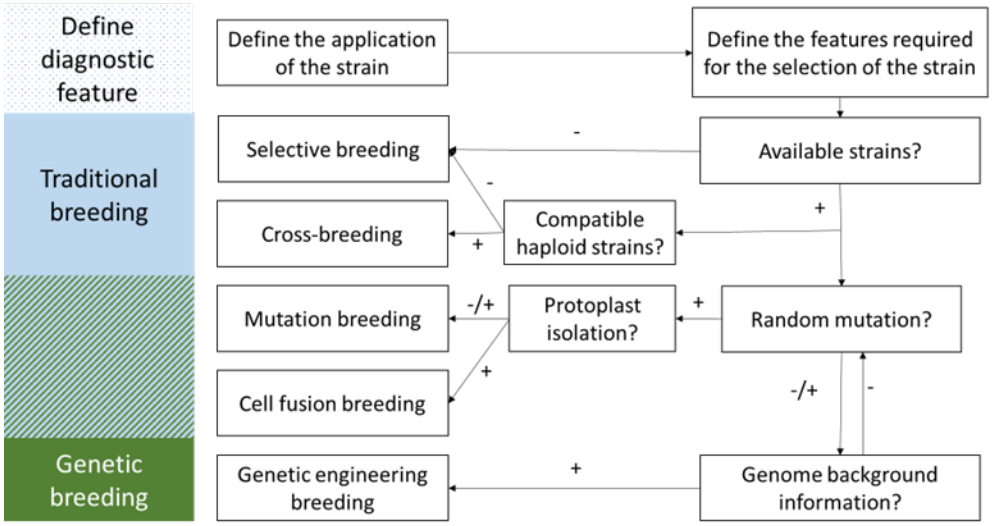

Figure 3 Breeding strategies to enhance the phenotypic or genotypic performance of fungal strains (Zhou et al. 2012).

strains. The limitation of this technique for the cultivation of *Ganoderma* species is the difficulties in the process to obtain haploid isolates from spore germination (Zhou et al. 2012). To overcome this limitation, the patent CN101352144A describes an efficient and fast method to obtain a monokaryotic strain from dikaryotic mycelium of *G. lucidum* instead from spores, increasing the probability of success and the efficiency in the process (Qingyu 2009).

Cell-fusion breeding. This method allows the recombination of strains through protoplast fusion. Cell-fusion breeding allows the recombination of non-compatible strains even if those consist of different fungal species (Zhou et al. 2012). For instance, the patent CN1742555A describes a method that allows the combination of *G. lucidum* and *G. tsugae* through protoplast fusion to obtain a faster growing hybrid strain presenting higher temperature range tolerance (Hua 2006). However, one of the limitations of the cell-fusion breeding technique is that the mutagenesis is random (Zhou et al. 2012).

Mutation breeding. Chemical and/or physical mutagens are applied to the strains to enhance the phenotypic or genotypic performance (Dong et al. 2022). For example, Peng et al. (2016) applied lithium chloride chemical mutagen to protoplasts of *G. lucidum* to enhance the production of triterpenes and polysaccharides. Some of the disadvantages of these techniques are that the mutation is random, and the selection of the mutant is challenging (Zhou et al. 2012).

Genetic engineering breeding. Genome editing techniques are used to transfer or silence genes in fungal genomes to efficiently obtain a desired strain. The genetic engineering breeding requires of extensive background information on the fungal genome and to know the specific genes to be targeted (Zhou et al. 2012). The limitation of this technique is the scarce availability of genome data for cultivated fungal species, as it is the case with *G. lucidum* (Kües et al. 2015). The strict regulations regarding the use of genetically modified organisms in the food industry, and the challenges related to customer acceptability (Wunderlich and Gatto 2015) complicates the application of this breeding technique. Despite not being the preferable breeding technique for food applications, the genetic engineering

methods may be a good pathway to obtain selective bioactive compounds for other industrial applications (Kothe 2001).

In the mushroom industry, the breeding method is generally optimized to obtain new strains with certain features, and often require of the combination of different breeding techniques (Dong et al. 2022; Salazar-Cerezo et al. 2023).

Cultivation techniques using solid-state fermentation

Solid-state fermentation methods are broadly used for the commercial production of fruiting bodies of *Ganoderma* species, which are log cultivation and substrate or substitute cultivation techniques (Table 2; Figure 4).

The **log cultivation** method used to be popular in China, where logs of up to 1 meter length were used as substrate without the sterilization step (Chen 2002), resembling the natural growing conditions of the species. However, this method required of a long period to obtain fruiting bodies (up to 3 years; Chen 2002); therefore, short logs (i.e. Sukarno et al. 2004) are preferred to reduce the production time (Chen 2002). The general cultivation steps are as follow: cutting wood logs or wood stumps, sterilization of the logs (optional – depending on the log size and the available equipment of the cultivators), placing the logs in a sand, soil or sawdust bed to maintain the moisture in the logs, inoculating the logs with the *Ganoderma* spawn, fermentation of the wood log (control of humidity, light exposure and O_2 exchange), and harvesting the fruiting bodies (Boh et al. 2007). *G. lucidum* derived products obtained through log cultivation are considered of higher quality and are valued higher in the Southeast Asian market, but longer cultivation times (6-24 months from inoculation to primordia formation) and lower yields compared to substitute cultivation methods have been noted (Hapuarachchi et al. 2018).

The **substitute cultivation** method consists of the use of lignocellulosic fibres as substrate. The lignocellulosic fibres are generally derived from the forestry and agricultural industries, and the cultivation steps differ from the log cultivation technique as follow:

Figure 4 Research on cultivation methods of laccate *Ganoderma* in Finland using solid-state fermentation techniques: wood log cultivation trial held in the Natural Resources Institute Finland, Suonenjoki (left), and substitute cultivation method used in Article II (right). Pictures by Henri Vanhanen. Research conducted at the Natural Resources Institute Finland (Luke).

substrate preparation, addition of supplements, mixing, packing of the substrate in bags (i.e. Gurung et al. 2012) or bottles (i.e. Kim et al. 2005), sterilizing of the substrate, inoculation of the substrate with the *Ganoderma* spawn, fermentation, and harvesting of the fruiting bodies. The substitute cultivation method is the most spread in the mushroom cultivation industry; both the bag and the bottle packing systems are used at an industrial scale as there is automated lines for both processes (Zhou 2017). Most of the production of *G. lucidum* fruiting bodies is done using substitute cultivation methods (Boh et al. 2007). The *G. lucidum* strain (Articles I-III), the spawn type (Roy et al. 2015), the spawn doses (Veena and Pandey 2010), the addition of supplements (Mehta et al. 2014), the substrate (Sudheer et al. 2018a), the particle size and volume of the substrate, and the packing container (Gurung et al. 2012; Kim et al. 2005) are important factors to optimize the substitute cultivation of *G. lucidum*.

Spawn

Spawn refers to the pure culture of the fungal species that is going to be used for inoculation in the cultivation process. The spawn is used in the mushroom industry to ensure that the desired fungal strain colonizes the substrate or growing media efficiently (Veena and Pandey 2010) and reduces the contamination ratio of the substrates (Oei 2016). The occurrence of contaminants is due to the growth of fungi and bacteria, such as *Aspergillus* spp., *Bacillus* spp. (Gupta et al. 2020) and *Trichoderma* spp. (Tang et al. 2023), or insects (Zhou 2017). The contamination of the spawn or the substrate media hinders the mycelial growth, the production of fruiting bodies and the further use of the fungal-derived products.

To produce spawn, the mycelium of the fungal species is propagated in a sterile nutrient-enriched medium, which can be solid or liquid. Several solid medium types have been used as spawn for the solid-state cultivation of *Ganoderma* species, and those include cereal grains such as barley (Article II; Soh et al. 2021), sorghum (Ghafoor et al. 2024), rye, oat, rice bran (Roy et al. 2015) and wheat (Azizi et al. 2012), wood sawdust (Liu et al. 2017), and less frequently agricultural plant biomass residues (i.e. straw). Wood plugs have been also used as spawn carriers for the inoculation of wood logs and wood stumps (Chen 2002). The dose of the spawn added to the substrate media has an effect to the efficiency of the cultivation process; for instance, Veena and Pandey (2010) obtained a higher yield from 59.0 to 118.5 g/kg (fresh fruiting body per wet substrate) by increasing the spawn dose by 4%.

Another inoculation technique used for the solid-state and liquid-state cultivation of *G. lucidum* involves the production of liquid spawn, also called inoculum (i.e. Yang et al. 2023). Liquid spawn is prepared through liquid-state fermentation of nutritious medium (Yang et al. 2023). Automated liquid spawn inoculation technologies (i.e. Wang and Li 2012; Kim 2006) are commonly used in the cultivation of edible and specialty fungal species such as *Lentinus edodes* (Zhou and Li 2015). Compared to solid spawn, automated liquid inoculation lines reduce the contamination risks in the cultivation process, as well as ensures the quality and replicability of the process (Friel and McLoughlin 2000; Zhou et al. 2012).

Growth substrate

An optimal substrate is often associated to the yield of fruiting bodies or bioactive compounds as well as the fermentation time. The selection of the growth substrate for the cultivation of *G. lucidum* depends on the local available resources and the substrate preference of the strains. Sawdust and wood chips of broadleaf tree species are commonly used for the substitute cultivation of species belonging to the *G. lucidum* complex.

Table 2 Solid-state fermentation methods used for the cultivation of *Ganoderma lucidum*

Method	Substrate	Yield	BE (%)	Days [g]	Reference
Substitute cultivation *bag*	Wood sawdust, wheat bran, corn powder, gypsum	-	10-21	42-50 [h]	Liu et al. 2017
	Wood chips and sawdust, rice bran	60-119 g/kg[a]	15-30	-	Veena and Pandey 2010
	Dalbergia sissoo sawdust	120 g/kg[a]	34	41	Mehta et al. 2014
	Mangifera sp. sawdust	150 g /kg[a]	43	35	Mehta et al. 2014
	Populus sp. sawdust	100 g/kg[a]	29	48	Mehta et al. 2014
	Quercus robur sawdust, wheat bran	-	13-38	-	Lisiecka et al. 2015
	Swietenia mahagoni sawdust, wheat bran, CaCO3	235.2 g/kg[a]	8	9	Roy et al. 2015
	Didterocarpur turbinatus sawdust, rice bran, $CaCO_3$	110.4 g/kg[a]	4	19	Roy et al. 2015
	Wheat straw	205.1 g /kg[a]	-	-	Ghafoor et al. 2024
	Rice straw	-	6	-	Magday et al. 2014
Substitute cultivation *bottle*	*Quercus dentata* sawdust	68.6 g/L[b]	-	33	Kim et al. 2005
	Poplar sawdust	47 g/L[b]	-	37	Kim et al. 2005
	Oak sawdust, wheat bran	3.3-8.7 g/g[c]	-	32-43	Kim et al. 2001
Log cultivation	*Populus deltoides*	3.2 g[d]	-	60-65 [h]	Bijalwan et al. 2021
	Hardwood sp.	-	-	50-60	Chen 2002
	Shorea sp.	6.4-26.3 g[e]	-	34-122	Sukarno et al. 2004
	Paraserianthes falcataria	7.9-30.1 g[e]	-	96-125	Sukarno et al. 2004
	Artocarpus indica	-	-	55-65	Geetha et al. 2012

Footnote: a, fresh weight of fruiting bodies per wet weight of substrate; b, fresh weight of fruiting bodies per litre of substrate bottle; c, dry weight of fruiting body per dry weight of substrate; d, dry weight of the fruiting bodies in the 1st flush; e, fresh weight of the fruiting bodies; f, fresh weight of fruiting bodies per dry weight of substrate; g, cultivation days from inoculation to primordia initiation; h, cultivation days from inoculation until harvest.

There is a wide range of tree species, including softwood species, that have been successfully used as substrate for the cultivation of *G. lucidum* such as wood sawdust from *Acacia* spp., *Acer* spp. *Alnus* spp., *Betula* spp., *Carpinus* spp., *Castanea* spp., *Dalbergia* spp., *Dipterocarpus* spp., *Fagus* spp., *Fraxinus* spp., *Gmelina* spp., *Jacaranda* spp., *Juglans* spp., *Malus* spp., *Mangifera* spp., *Melia* spp., *Populus* spp., *Pyrus* spp., *Quercus* spp., *Robinia* spp., *Shorea* spp., *Swietenia* spp., and others (Azizi et al. 2012; Gurung et al. 2012; Mehta et al. 2014; Yu et al. 2017; Amiri-Sadeghan et al. 2022). Side-streams from the agricultural industry are also used as substrate for the substitute cultivation of *G. lucidum* such as cotton

stalk, wheat straw, mesocarp fiber from oil palm, sunflower seed hulls, and rice straw, husk, and bran (Yu et al. 2017; Sudheer et al. 2018a; Bidegain et al. 2019; Rashad et al. 2019).

The growing substrates are often mixed with other ingredients to optimize the cultivation process, and these are referred as supplements. Supplements that have been reported to increase the fruiting body yield and/or promote the growth of *G. lucidum* are often derived from the food industry such as rice bran, wheat bran, rye, ground soy, tea wate, $CaCO_3$, corn gluten, and potato dextrose (Kim et al. 2005; Gurung et al. 2012; Soh et al. 2021). For instance, Mehta et al. (2014) observed higher yields and lower fruiting body production times when they combined *Dalbergia sissoo*, *Mangifera* sp. and *Populus* sp. sawdust substrates with different ratios of wheat bran, corn flour and rice bran supplements.

The versatility of the *G. lucidum* to grow in a wide range of substrate types allow the valorisation of several by-products from agricultural and forestry industries to produce fungal-derived products. In Finland, the forestry sector produces large volumes of wood by-products (Hassan et al. 2018). The most cultivated tree species in Finland are *Pinus sylvestris*, *Picea abies*, and *Betula* spp.; by-products from those tree species could also be used as substrates for the cultivation of *G. lucidum*. On this dissertation, the suitability of by-products from the Finnish forest industry was tested as substrate to produce fruiting bodies of *G. lucidum* and to enhance the production of *β*-glucan.

Taxonomy of the *Ganoderma lucidum* complex

The correct identification of fungal species is essential for any mycological research such as fungal biodiversity and fungal biotechnology. To introduce the cultivation of *G. lucidum* in a geographical region is important to investigate both the physiology and the genetic variability of the local natural populations of the species. *Ganoderma* species have traditionally been differentiated based on morphological characteristics of the basidiocarp and the spores (Curtis 1781; Karsten 1881). However, these morphological characteristics are often very similar between different *Ganoderma* species, which complicates the delineation of species.

The *Ganoderma* species occurring in Europe are *G. adspersum* (Schulzer) Donk, *G. applanatum* (Pers.) Pat., *G. carnosum* Pat., *G. lucidum* (Curtis) P. Karst., *G. pfeifferi* Bres., *G. resinaceum* Boud and *G. valesiacum* Boud (Pristas et al. 2023). The phenotypic heterogeneity among *Ganoderma* species makes the taxon identification difficult when morphology is used as the basis for species identifications, such as the case of *G. lucidum*. *G. lucidum* was described by Curtis (1781) by using a specimen collected from London. The holotype specimen was lost and only an illustration of the basidiocarp is available (Curtis 1781), complicating the correct identification of the species. *G. lucidum* is often referred to as species complex due to the morphological similarities with several species (Adaskaveg and Gilbertson 1988; Moncalvo et al. 1995; Zhou et al. 2015). These morphologically similar species include *Ganoderma curtisii*, *Ganoderma japonicum*, *G. lucidum*, *Ganoderma neojaponicum*, *Ganoderma oregonense*, *Ganoderma tsugae* and *Ganoderma valesiacum*.

Besides the morphological features, the host-tree species has been another characteristic used for traditional species identification in the *Ganoderma* genus (Curtis 1781; Karsten 1881). Field and culture studies have, however, shown that many *Ganoderma* spp. are not limited to a specific host-tree species (Jahn et al. 1980; Adaskaveg and Gilbertson 1988), making host associations as a differential characteristic problematic. For instance, *Ganoderma lucidum* (Curtis) P. Karst. was for some time considered to cause decay only in

deciduous trees, therefore, other names were used for laccate *Ganoderma* occurring on conifers in Europe.

With the development of laboratory and molecular techniques, the correct identification is becoming more accurate allowing the delimitation between close related species. The DNA regions applied for phylogenetic analysis in the *G. lucidum* complex are limited to few genes or partial genes (Sun et al. 2022a; see Table 3). The study of a single DNA region for taxonomic purposes does not resolve the phylogeny of the *G. lucidum* complex; for instance, Hseu et al. (1996) reported that random amplified polymorphism DNA marker (*RAPD*) grouped isolates belonging to the *G. lucidum* complex differently to the internal transcribed spacer (*ITS*) dataset. Despite the advance in molecular techniques, there is still lack of multi locus sequence data available from type specimens and from different geographical areas. The unavailability of sequence data complicates the phenotypical boundaries and geographical range of different *Ganoderma* taxa, especially in the case of the *G. lucidum* complex.

Ganoderma lucidum in Finland

Ganoderma genus was described by P. A. Karsten in 1881, and the only species included in the genus was *G. lucidum* (Karsten 1881). Karsten (1889) reported the occurrence of *G. lucidum* in Ruissalo, Merimasku and Vaasa areas (Finland) growing on *Quercus robur*, *Alnus* sp. and *Picea abies* tree stumps. Up to date, only two *Ganoderma* species have been reported natively from Finland, *G. applanatum* (sub-genus *Elfvingia*) and *G. lucidum* (sub-genus *Ganoderma*) (Niemelä 1982; Niemelä and Kotiranta 1986). The *Ganoderma* specimens with laccate fruiting bodies have been identified as *G. lucidum*, and the non-laccate basidiocarps have been referred as *G. applanatum*.

Ganoderma applanatum is commonly distributed throughout Finland and it can be found in a wide spectrum of tree hosts such as *Alnus* sp., *Betula* spp., *Populus* spp., *Quercus robur*, *Salix* sp., and several other tree species (Niemelä and Kotiranta 1986). *Ganoderma lucidum* has a rare occurrence in Finland, and it has been found in stumps of *Alnus glutinosa*., *Betula* sp., *Larix* sp., *P. abies*, *P. sylvestris*, and *Quercus robur* (Niemelä and Kotiranta 1986).

Regarding the Finnish *G. lucidum* material, there is no robust DNA sequence data except for few ITS sequences publicly available in GenBank. Moreover, there has not been any phylogenetic studies concentrated in *G. lucidum* originating from Finland or comparing these regional strains to *G. lucidum* from other geographical locations. The taxonomy positioning of Finnish laccate *Ganoderma* species is relevant for future biotechnological and commercial application of the fungi because it allows a better understanding of the relation with related fungal species, and it aids in defining and comparing the secondary metabolite and bioactivity research data of the *G. lucidum* complex. Furthermore, the identification of the species is a regulatory requirement to commercialize products derived from fungi.

Table 3 DNA regions used in phylogenetic studies to identify species belonging to the *Ganoderma lucidum* complex

DNA region	References
Internal transcribed spacer (*ITS*)	Moncalvo et al. 1995, Guglielmo et al. 2007 and 2008, Matheny et al. 2007, Wang et al. 2009, Park et al. 2012ab, Cao et al. 2012, Zhou et al. 2015, Hennicke et al. 2016, Kwon et al. 2016, Jargalmaa et al. 2017, Zhang et al. 2017, Loyd et al. 2018ab, Xing et al. 2018, Sun et al. 2022a, **Article I**, **Article IV**
Partial β-tubulin (*β-tub*)	Park et al. 2012ab, Hennicke et al. 2016, **Article IV**
Elongation factor 1-α (*tef1*)	Cao et al. 2012, Zhou et al 2015, Jargalmaa et al. 2017, Loyd et al. 2018ab, Xing et al. 2018, Sun et al. 2022a, **Article IV**
RNA polymerase II subunit (*rpb2*)	Matheny et al. 2007, Cao et al. 2012, Zhou et al. 2015, Jargalmaa et al. 2017, Loyd et al. 2018a, Xing et al. 2018, Sun et al. 2022a, **Article IV**
Amplified fragment length polymorphism (*AFLP*)	Qi et al. 2003, Zheng et al. 2009, Wu et al. 2009
Large subunit of nuclear ribosomal RNA (*nLSU*)	Moncalvo et al. 1995, Sun et al. 2022a
Mitochondrial small-subunit ribosomal DNA (*mt-SSU*) and nuclear ribosomal RNA (*nSSU*)	Zhou et al. 2015, Sun et al. 2022a
Whole genome	Binder et al. 2013, Cortina-Escribano et al. 2020c, Sun et al. 2022b,

MATERIALS AND METHODS

Fungal strains

The fungal strains of laccate *Ganoderma* fruiting bodies resembling *G. lucidum* were collected between 2016 and 2018 from North Ostrobothnia, Satakunta and Uusimaa provinces in Finland. The strains were collected by the Natural Resources Institute Finland staff. Also, some of the strains were provided by citizens after a public announcement from the Natural Resources Institute Finland aimed at collecting strains from the Finnish forests.

The host species of the *Ganoderma* specimens were *Picea abies* and *Betula pubescens* wood stumps. The strains were isolated from the fruiting bodies by transferring a piece of

tissue from the basidiocarps context to 2% malt extract agar [MEA: 20 g malt extract/l (VWR International, LLC, USA), 15 g agar bacteriological/l (VWR Chemicals, LLC, USA)]. To obtain pure cultures of the strains, the growing margins of the colony were transferred to new plates several times. The fungal strains were preserved on 5% MEA agar at 5 ˚C in the Culture Collection of Natural Resources Institute Finland (Luke), Joensuu and Helsinki, Finland. The strains were used in the strain comparison, cultivation, and phylogenetic studies (Articles I, II, III, and IV).

Substrate media

Wood by-products from *Alnus incana*, *Betula* spp., *Larix* sp., *Populus tremula*, *Picea abies*, and *Pinus sylvestris* were used as substrate for the cultivation of *Ganoderma lucidum* originating from Finland. The wood by-products from *Betula* spp. consisted in a mixture of wood chips from *Betula pendula* and *Betula pubescens*. The wood chips (particle size from 20 to 50 mm) and sawdust (ca. 0.8 mm particle size) from the above tree species were obtained from plywood and sawmill companies located in North Karelia and South Savo regions in Finland. The wood by-products were preserved at -20 ˚C.

The lignocellulosic composition (extractive, carbohydrate, lignin, and ash content) of the *P. tremula* and *P. sylvestris* wood substrates was analysed. The methodology used is described in Article III.

Cultivation process

The cultivation process consisted of the following steps: agar transfer, spawn preparation and inoculation, substrate preparation and inoculation, fermentation, cold shock treatment, fruiting body formation, and harvest. The cultivation process from the agar production until the cold shock treatment took place in the laboratory facilities of the Natural Resources Institute Finland (Luke), Joensuu, Finland. The latest cultivation stages, fruiting body formation and harvest, were conducted in green house facilities at Botania (Joensuu), and the Natural Resources Institute Finland (Luke), Punkaharju, Finland.

The spawn was prepared by using grains of barley, oat, and rye. The grains were boiled for 1–3 hours, or soaked overnight, strained with a sieve, and finally autoclaved for at 125 ˚C and 1.2 bar in polypropylene bags. After cooling, the sterilized grains were inoculated with agar pieces containing actively growing mycelium of the laccate *Ganoderma* strains. The bags were mixed, sealed, and kept at 22 ˚C in dark conditions until full colonization. The colonized grain bags are referred later as spawn.

Polypropylene bags were filled with 1 kg (dry weight) of wood substrate. The moisture content (dry basis) of the substrates was adjusted to 65-75%. The substrate bags were sterilized in an autoclave at 125 ˚C and 1.2 bar and inoculated with spawn. The inoculated bags were mixed, sealed, and kept at 22 ˚C in dark conditions during the fermentation phase for approximately 6 to 8 weeks. When the mycelium colonized the substrate, the bags were subjected to 2 different cold shock treatments to induce primordia formation. The treatments consisted of exposing the bags to +5 ˚C and -20 ˚C. Then, the substrate bags were transferred to a greenhouse with a relative humidity of 65 to 75% and light exposure (8/24 h) until the fruiting bodies were fully grown and harvested, which took approximately one to two weeks from primordia formation until fruiting body harvest.

Mycelial growth

The mycelial growth was determined by following the radial growth of the colony on agar plates. The radial growth measurements were done every two days since the inoculation of the agar plates. The agar medias used to evaluate the mycelial growth were the following: malt extract agar (MEA) consisting of 20 g malt extract/L, 15 g agar bacteriological/L; potato dextrose agar (PDA) consisting of 20 g potato dextrose agar/L; water agar (WA) consisting of 15 g agar bacteriological/L; and wood amended media consisting of 20 g/L of dried wood sawdust from *Betula* spp., *P. tremula*, *P. abies*, *P. sylvestris* and *Larix* sp. added to the agar media (MEA, PDA and WA).

Fruiting body yield and *β*-glucan content

The yield was determined based on the fresh weight (g) of the cap and stipe of the fruiting bodies at the time of harvesting. After harvesting, the fruiting bodies were freeze-dried kept at -20 ˚C. The *β*-glucan content of the freeze-dried fruiting bodies were measured according to McCleary and Draga (2016). Prior to the glucan analysis, individual fruiting bodies were cut into pieces and milled (1 mm). Two samples were measured from each fruiting body. Total glucan, α-glucan and *β*-glucan content were reported as a percentage of the freeze-dried fruiting body (g/100 g).

Morphological features

The morphological characteristics of the mycelium colony were observed when the agar media was fully colonized or when the mycelial growth stopped (Article I). The criterion used to examine the morphological characteristics of the mycelium was based on the methodologies described by Sobal et al. (2007) and, Engelkirk and Duben-Engelkirk (2008). The macromorphological features of the cultivated fruiting bodies were observed after harvesting. The dimensions of the cap and stipes, the colour of the caps, and the presence of insect, fungi, or other type of contamination were recorded.

Phylogenetic analysis

DNA extraction, PCR and sequencing

DNA was extracted from the fungal strains using PrepMan Ultra Sample (Applied Biosystems, Fosters City, CA, USA). The gene regions amplified were the internal transcribed spacer (*ITS*), and portions from genes encoding for transcription elongation factor 1A (*tef1*), β-tubulin (*β-tub*) and RNA polymerase II (*rpb2*). The forward and reverse primers used to amplify the gene regions are shown in Table 4.

Table 4 Primers used to amplify the *ITS*, *tef1*, *β-tub* and *rpb2* regions

Primer	Sequence	Reference
ITS1-F	5'-CTT GGT CAT TTA GAG GAA GTA A-3'	Gardes and Bruns 1993
ITS4	5'-TCC TCC GCT TAT TGA TAT GC-3'	White et al. 1990
EF595F	5'-CGT GAC TTC ATC AAG AAC ATG-3'	Kauserud and Schumacher 2001
EF1160R	5'-CCG ATC TTG TAG ACG TCC TG-3'	Kauserud and Schumacher 2001
β-tubF	5'-CCG GTG CAG GCA TGG GTA CC-3'	Park et al. 2012
β-tubR	5'-TGA AGA CGG GGG AAG GGA AC-3'	Park et al. 2012
G-RPB2-F1	5'-CAT CGA GTT CTT GGA GGA GTG G-3'	Cao et al. 2012
G-RPB2-R1	5'-CGG AAT GAT GCT GGC ACA GAC A-3'	Cao et al. 2012

Table 5 Thermal cycling protocol

Initial denaturation	95 ˚C	3 min	1 cycle
Denaturation	95 ˚C	30 sec	
Annealing	52 ˚C	30 sec	35 cycles
Extension	72 ˚C	1 min	
Final extension	72 ˚C	10 min	1 cycle
Hold	4 ˚C	∞	

The PCR mixes were prepared per each forward and reverse primer as described in the Article IV. The PCR mixes were exposed to a thermal cycling protocol as indicated in Table 5. The PCR products were purified with exo SAP protocol described in Article IV. The sequencing reactions of the purified PCR products were performed using BigDye® Terminator v3.1 Ready Reaction mixture (Perkin-Elmer Applied Biosystems, Warrington, UK) following the protocol of the manufacturer. The PCR products were precipitated, dried and sequenced with an ABI Prism 3100 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) at the University of Pretoria.

The forward and reverse sequences of each primer were assembled in Geneious 102.6 (Biomatters Ltd, Auckland, New Zealand), and the consensus sequences were submitted and published in GenBank.

Sequence and phylogenetic analyses

Four different datasets were created and edited using Molecular Evolutionary Genetic Analysis (MEGA) v. 10.0.5 (Kumar et al. 2018) corresponding with each gene regions sequenced (*ITS*, *tef1*, *β-tub*, and *rpb2*). The datasets included sequences of species belonging to the *Ganoderma lucidum* complex and related species, namely: *G. adspersum*, *G. annulare*, *G. applanatum*, *G. australe*, *G. boninense*, *G. curtisii*, *G. flexipes*, *G. gibbosum*, *G. lingzhi*, *G. lucidum*, *G. mirabile*, *G. multipileum*, *G. oregonense*, *G. resinaceum*, and *G. sichuanense*. The datasets were aligned with FFT-NS-1 (*ITS* dataset) and G-INS-I (*tef1*, *β-tub*, and *rpb2* datasets) strategies using MAFFT v.7 (Katoh and Stanley 2013). The phylogenetic analyses consisting of Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) methods are described in more detail in the Article IV.

RESULTS AND DISCUSSION

Cultivation of *Ganoderma lucidum* in Finland

Breeding strategy

This study is the first to explore the substitute cultivation of laccate *Ganoderma* strains originating from the Nordics and, therefore, there is no previous reports on breeding techniques for the cultivation of Finnish strains belonging to the *G. lucidum* complex. One of the limitations of applying different breeding strategies is the scarce availability of local Finnish strains. All the strains screened in this thesis have been isolated from wild fruiting bodies growing on wood stumps of *Betula pubescens* and *Picea abies* from Finnish forests. Due to the limited genetic and phenotypic background of the Finnish populations of *G. lucidum*, a traditional selective breeding strategy was considered the most appropriate technique to explore the cultivation of the species.

Given the initial stage of commercial *G. lucidum* substitute cultivation in Finland, emerging mushroom producers would need to create their own pool of strains isolated from Finnish forests or use available commercial strains of *G. lucidum*. In the mushroom industry, it is common to use commercial strains to reduce the variation of the quality and yield of fungal-derived products (Oei 2016). However, growers and researchers continuously breed and select new strains to enhance the performance of existing strains and to improve the biotechnological process (Zhou et al. 2012). Regarding the use of commercial *G. lucidum* strains for mushroom cultivation, there are no Finnish strains commercially available. Prior cultivating commercial strains originating from other geographical regions, there are some aspects that should be taken into consideration (see the section 4.2.1).

The selective breeding in this study provided background information on the performance of the isolated Finnish strains belonging to the *G. lucidum* complex and enabled the further selection of a potential strain aimed for cultivation and production of fungal derived *β*-glucan. Several strains from the Natural Resources Institute Finland (Luke) fungal culture collection were screened in different growing media, substrate composition, and growing conditions. The selective breeding of the strains was optimized to enhance the defined features: mycelial growth (Article I), probability of fruiting, fruiting body yield, and *β*-glucan content in the fruiting bodies (Article II) and in the mycelium. Moreover, the selective breeding scheme used aimed to compare the strains in regards of their capability to grow on different wood-based substrate (Articles I-III).

The scarce availability of Finnish *G. lucidum* strains presents a challenge for the application of breeding techniques such as cross-breeding; however, other techniques could be used to obtain efficient strains for cultivation and *β*-glucan production. For instance, Ma et al. (2018) obtained *G. lingzhi* strains by plasma mutagenesis that presented higher growth rates and the polysaccharide content in the mycelium was increased by 25.6% compared to the parental strain. Therefore, the mutation breeding technique could be also explored in the best performing Finnish strains to enhance the production of *β*-glucans. Also, the genetic engineering breeding could be suitable to obtain *G. lucidum* strains with enhanced polysaccharide (Li et al. 2016) and secondary metabolite (Zhou et al. 2024) production. However, strains obtained from genetic engineering breeding may require the genetically modified organism certification (Wunderlich and Gatto 2015), which present an obstacle for their commercialization. Therefore, the development of genetic engineered strains is not

recommended for the mushroom industry in the Nordic countries. The selective breeding technique would be preferred to initiate the breed of strains and to create a larger phenotypic and genetic pool of *G. lucidum* in Finland.

Strain selection

The strain selection depends on the diagnostic features and desirable traits that the strains should present for a successful biotechnological process. In this thesis, the diagnostic features have been focused on the capabilities of the strains to produce fruiting bodies and polysaccharides. Moreover, the growing speed and the flexibility of the strains to grow in different substrate have also been used as diagnostic features in the breeding process. The strains performed differently considering all the diagnostic features evaluated in this work (Table 6). The *G. lucidum* strains presented differences in their **mycelial growth** and morphology growing under the same conditions and medium composition (Table 7). The mycelial growth of all the strains was generally enhanced when adding 2% of malt extract or potato dextrose in the agar medium. In MEA, the mycelial growth rate of the Finnish *G. lucidum* strains ranged from 0.30 to 0.53 cm/day. Similar mycelial growth rates of *G. lucidum* growing in MEA and PDA have been reported by Hernández-Rosas and Sánchez Meraz (2021). However, higher mycelial growth rates are frequently observed for *G. lucidum* among the literature (Badalyan et al. 2015; Ofodile et al. 2022; Ghafoor et al. 2024). For instance, Ghafoor et al. (2024), cultivated *G. lucidum* strains originating from Pakistan and reported a mycelial growth of 1.2 cm/day in PDA.

The mycelium growth rate on agar medium was not associated to the successful cultivation of *G. lucidum*. Among the Finnish *G. lucidum* strains, MUS9 presented the lowest mycelial growth in agar medium regardless of the addition of malt extract or potato dextrose. Still, MUS9 presented a higher probability of fruiting than MUS12, which had the highest mycelial growth rate on agar. Similar was observed by Owaid et al., (2015), who did not find a correlation between mycelial growth and fruiting body occurrence for *Pleurotus* spp. Despite not being an optimal indicator for fruiting body formation, the mycelial growth rate is an important diagnostic feature to compare the performance of strains under different growing conditions, such as different pH (Yang and Liau 1998), temperature (Badalyan et al. 2015) or sugar supplementation (Ghafoor et al. 2024).

Table 6 Diagnostic features to determine the performance of *Ganoderma lucidum* strains

Strain	Mycelial growth[a]	Probability of fruiting[b]	Fruiting body yield[c]	*β*-glucan content (%)	Substrate preference[d]
MUS6	0.45 ± 0.05	60	47.9 ± 15.0	52.0 ± 0.3	*Betula* sp.
MUS9	0.30 ± 0.04	47	43.5 ± 13.1	53.9 ± 0.4	*Betula* sp.
MUS12	0.53 ± 0.08	30	44.0 ± 13.1	49.5 ± 0.6	*P. tremula*
MUS19	0.50 ± 0.03	100	37.2 ± 7.9	51.2 ± 0.4	*P. tremula*
MUS75	0.33 ± 0.03	13	27.4 ± 9.0	55.9 ± 0.7	*P. tremula*
MUS192	0.41 ± 0.03	67	51.3 ± 18.7	53.9 ± 0.3	*P. tremula*

Footnote: a, mycelial growth (cm/day) in MEA medium determined after 12 days from inoculation; b, probability of fruiting when cold shock treatment at +5˚C; c, fresh weight (g) of the fruiting bodies per dry weight (kg) of substrate in the first flush; d, determined according to the yield performance.

The **probability of fruiting** was determined for different *G. lucidum* strains, substrate combinations, and fruiting body induction treatments, previously referred as cold treatments (Article II). All the strains presented difficulties to develop fruiting bodies. Primordia formation was not observed after 2 months of fermentation process since the full colonization of the substrate. During the cultivation of Finnish *G. lucidum* strains, a cold shock treatment was implemented to trigger the fruiting body initiation. The cold shock treatment aims to mimic the natural climatic conditions that the fungal species needs to develop fruiting bodies (Mui 2002). A cold shock treatment has shown to induce fruiting body production in the cultivation process of *Lentinula edodes* and *Pleurotus* sp. (Tarushi and Sud 2022; Ibrahim et al. 2015). The probability of fruiting increased by 89% when a cold shock treatment of 5˚C was applied to the *G. lucidum* colonized substrate bags. However, dropping the temperature bellow 0˚C is not recommended as the probability of fruiting was reduced to 10.5% when a cold shock treatment of -20˚C was applied. These results suggest that dropping the temperature to a frozen state will interrupt the growth cycle of *G. lucidum* and, when exposed to fruiting conditions (20-25˚C; RH 60-85%), the substrate will be prone to other organism competition. The colonized substrate bags exposed to -20˚C presented contamination 2 to 3 weeks after exposing them to fruiting environmental conditions.

The probability of fruiting was also affected by the strain used in the cultivation process (Table 6). All the substrate types inoculated with MUS19 strain and exposed to a cold shock treatment of 5˚C produced fruiting bodies. The strains MUS12 and MUS75 presented a low probability of fruiting and were able to develop fruiting bodies with limited tree species.

In mushroom cultivation research, the **fruiting body yield** is often reported as biological efficiency. The biological efficiency measures the capacity of a certain strain to transform a substrate into fruiting bodies expressed as the fresh fruiting body weight per dry weight of the substrate. In this study, the biological efficiency of the Finnish *G. lucidum* strains was low compared to previous reports using different strains and substrates (Amiri-Sadeghan et al. 2022). Previous reports indicate a wide range on the biological efficiency of *G. lucidum*, varying from 0.81% to 68.35% (Amiri-Sadeghan et al. 2022). There are no reports on a specific biological efficiency considered optimal for *G. lucidum*; however, most of the reported biological efficiencies compiled in a literature review by Amiri-Sadeghan et al. (2022) fall within a range between 8.5 to 38.5%. In this study, the Finnish strains presented a biological efficiency ranging from 3.72 to 5.13% for MUS19 and MUS192 respectively (Table 8). The substrate composition should be optimized to enhance the fruiting body yield of Finnish *G. lucidum*.

Table 7 Mycelial growth (cm/day) of *Ganoderma lucidum* in agar media

Strain	Control	PDA (2%)	MEA (2%)
MUS6	0.1 ± 0.01	0.37 ± 0.04	0.45 ± 0.05
MUS9	0.14 ± 0.02	0.18 ± 0.04	0.30 ± 0.04
MUS12	0.09 ± 0.05	0.50 ± 0.12	0.53 ± 0.08
MUS19	0	0.14 ± 0.06	0.50 ± 0.03
MUS75	0.06 ± 0.04	0.31 ± 0.12	0.33 ± 0.03
MUS192	0.19 ± 0.01	0.25 ± 0.03	0.41 ± 0.03

Table 8 Biological efficiency (%) and *β*-glucan content (%) of *Ganoderma lucidum* strains originating from Finland on different wood-based substrates

	Betula spp.		*P. tremula*		*P. abies*		*P. sylvestris*		*Larix* sp.	
Strain	BE	β-glu	BE	β-glu	BE	β-glu	BE	β-glu	BE	β-glu
MUS6	9	53.5	6	54.8	5	51.4	3	48.8	2	52.7
MUS9	7	54.8	7	58.9	3	55.9	3	46.0	2	53.0
MUS12	4	52.4	5	54.3	-	-	2	42.1	-	-
MUS19	4	51.2	4	54.2	3	51.8	4	48.9	1	50.1
MUS75	-	-	3	55.9	-	-	-	-	-	-
MUS192	7	54.4	9	56.6	5	52.5	3	50.7	2	54.9

The overall ***β*-glucan content** in the fruiting bodies of the Finnish strains was high, varying from 42.1 to 58.9% (Table 8; Article II). Similar levels were observed by McClearly and Draga (2016). Lower levels were reported by Cho et al. (2013), who obtained a *β*-glucan range of 15 to 20% in fruiting bodies of several *Ganoderma* species including *G. lucidum*. In another study, Kim et al. (2017), obtained the highest *β*-glucan content of 6.20 g/100g from *G. lucidum* fruiting bodies extracted with a pH of 10 and using water as solvent. Other cultivated species have shown lower *β*-glucan content in their fruiting bodies compared to the Finnish cultivated *G. lucidum*; for instance, Diamantopoulou et al. (2023) obtained a range of 18.23 to 44.14%, 20.24 to 49.95% and 8.02 to 13.91%, respectively for *Pleurotus ostreatus*, *Pleurotus eryngii* and *Agaricus bisporus*.

Effect of tree species on the cultivation performance

The potential of wood by-products from the forest industry was evaluated for the cultivation of *Ganoderma lucidum*. The tree species influenced the mycelial growth, the probability of fruiting, the fruiting body yield, the *β*-glucan content of the *G. lucidum* strains. Moreover, different delignification ratios by *G. lucidum* were observed for the tree species (Table 9).

To increase the **probability of fruiting** body production, the tree species selected as substrate is found essential. The strains had difficulties to colonize softwood species, especially *P. sylvestris* and *Larix* sp. wood-based substrates, and in several replicates the mycelial growth stopped. MUS75 and MUS12 did not produce fruiting bodies in substrates composed of *Larix* sp. and *P. abies* wood substrates (Table 8). On the other hand, *P. tremula* and *Betula* spp. were the best performing substrates for all the diagnostic features (Table 10).

The selection of the tree species had a significant effect on the ***β*-glucan content** of the *G. lucidum* fruiting bodies; the lowest was 47.5% and the highest 55.8% respectively for *P. sylvestris* and *P. tremula* (Table 9). Kuhar et al. (2018) also tested *Pinus* sp. and *Populus* sp., to produce *G. lucidum* fruiting bodies and obtained higher yields when using *Populus* sp. as substrate. To better understand the degradation process of the substrates by *G. lucidum*, the chemical composition of *P. tremula* and *P. sylvestris* was compared prior and after the cultivation of *G. lucidum* (Article III). Despite not being an optimal substrate for *β*-glucan production, *P. sylvestris* was successfully delignified by *G. lucidum*. On the other hand, the glucan fraction was not degraded in the case of *P. sylvestris* substrate. The wood chemical fractions of *P. tremula* were degraded evenly. These results suggest that the fermentation of

P. sylvestris by *G. lucidum* could be a precursor for other biotechnological applications (Blanchette 1991). To further understand the biotransformation of wood components into *G. lucidum* polysaccharides, it is recommended to study the correlation between the degradation of lignin, cellulose and hemicelluloses and the production of *β*-glucan in the fruiting bodies of *G. lucidum*.

There is a potential to use wood by-products as cultivation substrate for *G. lucidum* in Finland, but the cultivation method needs to be optimized. According to the cultivation trials of the present thesis, to cultivate *G. lucidum* in Finland it is recommended to first select a substrate consisting of *P. tremula* or *Betula* spp. wood by-products, and then select the best performing Finnish *G. lucidum* strain. Amendments to the substrate media composition would be needed to enhance the probability of fruiting and the fruiting body yield of Finnish *G. lucidum*. The addition of supplements to wood-based substrates have shown to increase the yield of fruiting bodies in *G. lucidum*. For instance, Mehta et al., (2014) increased the biological efficiency of *G. lucidum* from 28 to 45% by adding 20% of wheat bran to *Populus* sp. wood substrate. To increase the probability of fruiting and the yield of fruiting bodies of Finnish *G. lucidum* strains it is then recommended to test the addition of supplements to the best performing wood-based substrates *P. tremula* and *Betula* spp. The particle size of the substrate may also affect the gas exchange and the fruiting body production of *G. lucidum* (Kuhar et al. 2018).

Table 9 Effect of tree species on the cultivation of *Ganoderma lucidum*

Substrate	Mycelial growth[a]	Probability of fruiting[b]	Fruiting body yield[c]	*β*-glucan production[d]
Betula spp.	0.52	67	59.8 ± 16.8	53.1 ± 0.3
Larix sp.	0.39	43	18.1 ± 5.0	52.7 ± 0.5
P. tremula	0.52	90	52.1 ± 13.5	55.8 ± 0.3
P. abies	0.42	60	38.2 ± 8.8	52.9 ± 0.4
P. sylvestris	0.46	57	34.6 ± 7.8	47.5 ± 0.8

Footnote: a, mycelial growth (cm/day) in MEA medium determined after 12 days from inoculation; b, probability of fruiting when cold shock treatment at +5˚C; c, fresh weight (g) of the fruiting bodies per dry weight (kg) of substrate in the first flush; d, determined according to the yield performance.

Table 10 Tree species preference for Ganoderma lucidum strains originating from Finland based on the diagnostic features

Strain	Mycelial growth	Probability of fruiting	Fruiting body yield	*β*-glucan content
MUS6	*P. tremula*	*Larix* sp.	*Betula* spp.	*P. tremula*
MUS9	*Betula* spp.	*P. sylvestris*	*Betula* spp.	*P. tremula*
MUS12	*P. tremula*	*Betula* spp.	*P. tremula*	*P. tremula*
MUS19	*Betula* spp.	*P. tremula*	*P. tremula*	*P. tremula*
MUS75	*Betula* spp.	*P. tremula*	*P. tremula*	*P. tremula*
MUS192	*Betula* spp.	*Larix* sp.	*P. tremula*	*P. tremula*

Taxonomic evaluation of *Ganoderma lucidum* in Finland

The phylogenetic results indicated that all the laccate *Ganoderma* strains isolated from Finnish specimens resembling *G. lucidum* belonged to the same species. The *ITS*, *tef1*, *β-tub*, and *rpb2* sequences generated from the Finnish strains were nearly identical for all specimens collected in conifer and deciduous tree species (Article IV). Moreover, the morphological characteristics of the fruiting bodies are uniform for the Finnish material examined in this study despite of the host tree species.

The only laccate *Ganoderma* species previously reported in Finland is *G. lucidum* (Niemelä 1982; Niemelä and Kotiranta 1986). In the present study, the Finnish isolates were closer related to the *G. tsugae* lineage than to *G. lucidum* in *tef1*, *rpb2* and *β-tub* phylogenies (phylogenetic trees shown in Article IV). The *tef1*, *β-tub*, and *rpb2* phylogenies separated the European *G. lucidum* and the Finnish strains in different clades, the latter clustering together with *G. tsugae*. Previous phylogenetic studies also separated the North American *G. tsugae* from the European *G. lucidum* (Zhou et al. 2015; Loyd et al. 2018a; Sun et al. 2022b). Interfertility tests between *G. lucidum* isolates and North American *G. tsugae* are recommended to further clarify the boundaries between the species.

The *ITS* data did not delimit strains of Finnish *G. lucidum*, European *G. lucidum*, East Asian *G. lucidum*, North American *G. tsugae* and Chinese *G. tsugae* in distinct clades (*ITS* phylogenetic tree shown in Article IV). A previous phylogenetic study based on the mitochondrial small-subunit ribosomal gene region also grouped *G. tsugae* and *G. oregonense* isolates from North America with *G. valesiacum* and *G. lucidum* from Europe in the same clade (Hong and Jung 2004). Cao et al. (2012) conducted a phylogenetic analysis of *Ganoderma* spp. using the *ITS* sequences of several species from China and Europe. Their phylogenetic tree grouped isolates labelled as *G. tsugae* from China with *G. lucidum* from Europe. They therefore suggested that the strains labelled as *G. tsugae* in GenBank were wrongly identified.

A recent taxonomic revision of Ganodermataceae (Sun et al. 2022b) indicated that the analysis of the *ITS* region alone does not differentiate *Ganoderma* species. Previous studies have demonstrated that in *G. lucidum* complex, *tef1* and *rpb2* loci are more informative than the *ITS* region (Loyd et al. 2018a; Sun et al. 2022b).

A phylogenomic approach may further aid in resolving the taxonomy of taxa in the *G. lucidum* complex. The application of phylogenomics in fungi is a convenient solution due to the small size of the fungal genomes and the straightforward sequencing methods, which often result in the construction of highly supported trees from a super matrix or a super-tree (Prasanna et al. 2019). However, there are some barriers with phylogenomic analysis, such as missing data and modelling of the data, which can lead to systematic errors (Prasanna et al. 2019). In the case of the *G. lucidum* complex, the currently scarce availability of *Ganoderma* genomes in public datasets is a major constraint in following a phylogenomic approach.

The taxonomic resolution of the laccate *Ganoderma* species in Finland is directly affected by the need to assign a type specimen for *G. lucidum*. Unfortunately, the designation of a type specimen representing *G. lucidum* is not a straightforward task (i.e. Steyaert 1972). Therefore, the laccate *Ganoderma* species distributed in Finland maintains the nomenclature of *G. lucidum*. To determine the classification of laccate *Ganoderma* in Finland, it is recommended to examine more specimens and collect more molecular data from *G. lucidum*

from Northern Europe. Also, it is recommended to study the relationship between *G. tsugae* originating from North America and *G. lucidum* originating from Finland.

Implications for the mushroom industry

Ganoderma lucidum is not considered novel food in the European Union (European Commission 2024). The *G. lucidum* complex is a taxonomically complicated group of species, in which the Finnish laccate specimens belong according to the phylogenetic results (Article IV). In the present thesis, the laccate *Ganoderma* species distributed in Finland maintains the nomenclature of *G. lucidum*. Therefore, there should not be obstacles in the commercialization of its derived fruiting body food products within the European Union. The present work (Article I-III) has proven that local strains of *G. lucidum* are suitable for fruiting body and *β*-glucan production.

Considering the present literature and the phylogenetic results of this thesis (Article IV), it is recommended to use local strains of *G. lucidum* for the cultivation of the species in Finland. The utilization of unknown, unreliable sourced commercial strain, or *G. lucidum* strains from other geographical region may present threats to the biodiversity of Finnish forest.

CONCLUSION

The present work is the first report on the successful cultivation of *G. lucidum* in the Nordic countries using local strains and lignocellulosic resources. The first research on selective breeding, strain comparison, *β*-glucan content, and phylogenetic analysis of *G. lucidum* originating from Finland is reported in this dissertation.

The substitute cultivation of *G. lucidum* strains originating from Finland was successfully achieved. The strain selection, the substrate composition, and the cold shock treatment were found essential to produce *G. lucidum* fruiting bodies. The strain and wood substrate selection had a significant effect on the production of fungal-derived *β*-glucans in the fruiting bodies of *G. lucidum*.

Strains differences were observed in terms of mycelial growth, fruiting body production and content of *β*-glucan. Strain comparisons and suitability of growth conditions and substrates were found essential for optimizing the yield of fruiting bodies and bioactive compound *β*-glucan. Although *β*-glucan has been recognized by its functionality, the study of other primary and secondary metabolites derived from Finnish cultivated *G. lucidum* is crucial to understand its bioactive potential.

The optimal cultivation substrate was not found related to the tree host species of the fungal strain. The highest yield and *β*-glucan content of *G. lucidum* fruiting bodies was obtained when using wood by-products of *Betula* spp. and *P. tremula* as substrate. The solid-state fermentation of wood by-products of *P. sylvestris* by *G. lucidum* was not found a suitable cultivation method. However, the fermentation of *P. sylvestris* by *G. lucidum* allowed the delignification of the lignocellulosic biomass, which can be a bioprocess precursor for other uses such as pulp production. Different cultivation conditions need to be further studied to gain understanding and to improve the substitute cultivation of Finnish *G. lucidum* strains.

The phylogenetic results indicated that all the laccate *Ganoderma* strains belonged to the same species. According to the results and literature presented, the *G. lucidum* strains originating from Finland consist of a single taxon residing in the *G. lucidum* complex. The present work generated and published for the first-time multi-locus sequence data for *G. lucidum* isolates originating from Finland.

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