Prospects for using *Ganoderma lucidum* (Curtis) P. Karst. for biological control of phytopathogenic fungi

I N Pavlov¹²⁴, Y A Litovka¹², P V Makolova¹², A A Timofeev¹³, E A Litvinova²³ and R Kh Enazarov¹²³

¹Sukachev Institute of Forest, Siberian Branch Russian Academy of Sciences, 50/28 Akademgorodok, Krasnoyarsk, 660036, Russia
²Reshetnev Siberian State University of Science and Technology, 31, Krasnoyarsky rabochy prospect, Krasnoyarsk, 660037, Russia
³ Federal Research Center Krasnoyarsk Science Center of the Siberian Branch of the Russian Academy of Sciences, Akademgorodok, 50, Krasnoyarsk, 660036, Russia

⁴E-mail: forester24@mail.ru

Abstract. Antifungal, morphological and cultural properties of the Siberian and Abkhaz strains of the medicinal basidiomycete *Ganoderma lucidum* (Curtis) P. Karst were studied. A group of strains characterized by high growth rates *in vitro* on agar media (7.3-8.0 mm / day) and plant waste from timber processing (3.3-3.9 mm / day) was found. All strains of *G. lucidum* exhibited antifungal activity against phytopathogenic fungi; the degree of phytopathogen inhibition ranged from 10 to 58%.

Basidial rootrot fungi *Heterobasidion* and ascomycete fungi *Bipolaris, Alternaria, Fusarium* are the most sensitive to the presence of *G. lucidum*. The cultures most effectively limiting the phytopathogen development in the group of fast-growing Abkhaz strains (antifungal activity ranged from 21 to 58%) were identified. The high antifungal activity of strains on lignocellulosic substrates allows us to consider them as promising biocontrol agents for reducing the number, primarily, of basidial phytopathogens.

1. Introduction

Fruitbodies and submersed mycelium of the *Ganoderma* are widely used in oriental medicine in order to create the broad-spectrum prophylactic and medicinal preparations. Biologically active substances (statins, polysaccharides, β-glucans, ganoderic acids) having antiallergic, immunostimulating, hepatoprotective, antitumor and antiviral effects were isolated [1, 2]. Fungi of the genus *Ganoderma* have shown antibacterial activity against *Pseudomonas syringae* and *Bacillus subtilis* [3]; *Escherichia coli, Alcaligenes faecalis, Proteus vulgaris, Neisseria meningitidis, Bacillus cereus, Staphylococcus aureus* [4]; *Enterococcus faecalis, Klebsiella pneumoniae, Listeria monocytogenes* [5, 6]. The antifungal activity of *Ganoderma* against microscopic fungi is poorly understood [7, 8], whereas there appears to be a feasibility of using some fast-growing strains to effectively limit phytopathogenic fungi. Of particular interest is the biological control of rootrot (basidiomycete fungi *Armillaria* and *Heterobasidion*), which are practically insensitive to the action of the widespread biocontrol fungus *Trichoderma*. 
2. Materials and methods
The study objects were 15 strains of *Ganoderma lucidum* (Curtis) P. Karst. Strains were isolated from basidiomata found in Russia (Central Siberia) and Abkhazia. Cultural features and growth parameters (radial growth rate and growth coefficient) were studied on carrot agar (CA), malt extract agar (MEA) and Norkrans-medium at 23 °C [9]. Microscopic observations of cultures were made using an Olympus CX41 microscope (Olympus Co., Tokyo, Japan) and a scanning electron microscope Hitachi SU3500 (Hitachi, Tokyo, Japan). Species identification was confirmed by sequencing of genetic markers ITS1-ITS4 with the use of equipment from the Core Centrum “Innovative Technologies in Plant Protection” FSBSI VIZR (St. Petersburg–Pushkin), and the SB RAS Genomics Core Facility (ICBFM, Novosibirsk). DNA was isolated from the mycelium of pure cultures preliminarily grown on 2 % MEA for 7–14 days at 24 °C without illumination by the CTAB method [10]. The evolutionary history was inferred by using the maximum likelihood method and Kimura 2-parameter model [11].

To determine the antifungal activity, agar blocks with a seven-day culture of *Ganoderma* and the tested phytopathogen strain in the optimal developmental stage were placed on Petri dishes. For ascomycete fungi, 2 % MEA was used; for basidiomycetes – aspen and fir sawdust. The blocks were placed at a distance of 6 cm from each other. The inoculations were incubated at 23 °C for 7-10 days. Antifungal activity (%) was calculated by estimating the phytopathogen colony radius with *Ganoderma* strain. The radius of the phytopathogen colony on a similar substrate in a monoculture served as a control [4]. Pure cultures of phytopathogenic fungi were used as test objects: *Fusarium sporotrichoides* Sherb., *Fusarium oxysporum* Schldl., *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Alternaria alternata* (Fr.) Keissl., *Corinectria sp.*, *Armillaria borealis* Marxm. & Korhonen, *Heterobasidion annosum* (Fr.) Bref., *Heterobasidion abietinum* Niemelä & Korhonen.

3. Results
The investigation of microstructures has shown that all studied *Ganoderma* cultures form well-developed abundant septate hyphae with clamp connections and anastomoses. Generative hyphae were thin-walled, with numerous septa. The thickness of the hyphae varied from 1.5 to 4 µm. Lateral branching was frequent, at right and acute angles, the width of lateral hyphae varies from 0.5 to 3 µm. As the culture aged, round or ellipsoidal - shaped mycelial thickenings, as well as crystal-like structures on the hyphae surface take place. The species identification of the studied strains was confirmed by sequencing the sites of the ITS1-ITS4 genetic markers. The phylogenetic tree with the highest log likelihood (-3299.43) for the *G. lucidum* species used in the study is given in Figure 1. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories (+G, parameter = 0.2827)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 30 nucleotide sequences. There were a total of 750 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [12].

Screening of fast-growing strains *in vitro* was carried out in three nutrient media. The southern strains of *G. lucidum* isolated in the territory of Abkhazia were included in the group of fast-growing basidiomycetes. Colonization of the entire surface of the agar plate in a Petri dish (diameter 90 mm) was noted in average on the seventh day. On natural media (MEA and CA), a well-developed, dense, (in places fluffy) white mycelium was formed. On a synthetic medium (Norkrans-medium), the mycelium was scanty, loose, and cotton-like. The maximum radial growth rate was shown to be on CA (7.3-8.0 mm / day); the growth coefficient varied from 64 to 85. Strains isolated in Siberia (with a characteristic morphology of colonies) were included in the group of slow-growing ones. Aerial mycelium was low, velvety, with leathery colony being difficult to separate from the agar plate. The colony color was white, darkening as the culture aged (from beige to dark brown). The maximum growth rate being 1.3-1.6 mm / day on CA and PDA, and the growth coefficient 19-27.
Figure 1. Phylogenetic tree of *Ganoderma lucidum* constructed with ITS regions by maximum likelihood method.

All strains of *G. lucidum* have exhibited antifungal activity against the studied phytopathogenic fungi, however, the degree of activity varied significantly depending on the *Ganoderma* strain and the test culture (table 1, figure 2). The degree of phytopathogen inhibition ranged from 10 to 58 %. Higher antifungal activity parameters were noted in the Abkhaz strains of *G. lucidum* (from 15 to 58 %) and individual Siberian strains (from 17 to 40 %). The most sensitive phytopathogens appeared to be basidiomycetes *Heterobasidion* (maximum growth inhibition was 55 %) and ascomycete fungi *Bipolaris* (inhibition was up to 58 %), *Alternaria* (up to 54 %), and *Fusarium* (up to 45 %). The root pathogen *Armillaria borealis* and the stem pathogen *Corinectria sp.* were relatively resistant to the *G. lucidum*.

Table 1. Maximum antifungal activity (%) of Siberian and Abkhaz strains of *Ganoderma lucidum*.

| Test object              | The Abkhaz strains | The Siberian strains |
|--------------------------|--------------------|----------------------|
|                          | Gl1-16A            | Gl2-16A              |
|                          | Gl3-16A            | Gl4-16A              |
|                          | Gl5-16A            | Gl6-17S              |
|                          | Gl7-17S            | Gl9-17S              |
|                          | Gl10-16S           | Gl11-16S             |
| *Alternaria alternata*   | 30                 | 24                   |
| *Armillaria borealis*    | 18                 | 19                   |
| *Bipolaris sorokiniana*  | 20                 | 29                   |
| *Corinectria sp.*        | 17                 | 15                   |
| *Fusarium oxysporum*     | 28                 | 29                   |
| *Fusarium sporotrichioides* | 29             | 34                   |
| *Heterobasidion abietinum* | 33               | 38                   |
| *Heterobasidion annosum* | 37                 | 41                   |
Figure 2. Antifungal activity of *Ganoderma lucidum* against the ascomycete (A) and basidiomycete (B) phytopathogens.

The strains of *G. lucidum*, which effectively inhibit phytopathogens, relatively quickly colonized mono- and mixed plant substrates (wood processing waste). The radial rate varied in the range of 3.3-3.9 mm / day, with the growth coefficient being 42-59. The antifungal activity of *G. lucidum* on plant substrates against the root rot pathogen *Heterobasidion* was comparable to that against ascomycete fungi on agar media.

4. Conclusion

The most promising biocontrol agents are Abkhazian strains of *Ganoderma lucidum*. They are characterized by maximum antifungal activity against phytopathogenic basidiomycete and ascomycete fungi (up to 58 % growth inhibition) on various growth substrates. The advantage of these strains is their high growth parameters on agar media and plant substrates (which is not typical for most basidiomycetes *in vitro*). The high growth rate of biocontrol strains opens up wide possibilities for their cultivation in various biotechnological systems, including on lignocellulosic substrates.

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