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Production of Polysaccharides With Antiphytoviral Activity by Ganoderma Applanatum and Ganoderma Lucidum: A Comparative Study

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Abstract

The higher basidiomycetes Ganoderma applanatum (Pers.) Pat. and Ganoderma lucidum (Curtis) P. Karst were compared for their ability to produce structural (intracellular) and extracellular polysaccharides (IPS and EPS) during submerged cultivation in a glucose-peptone-yeast extract medium. Polysaccharide fractions isolated from mycelium and culture liquid were tested for antiviral activity against tobacco mosaic virus (TMV) using three indicator plants differing in virus localization capacity: Datura stramonium L. (high), Chenopodium amaranticolor Costa et Reyn. (moderate), and Nicotiana sylvestris L. (low). G. lucidum and G. applanatum differed substantially in biomass accumulation and polysaccharide production during submerged cultivation in a medium containing 20 g L^{-1} glucose. G. lucidum accumulated substantially more biomass (21.5 g L^{-1}) than G. applanatum (3.8 g L^{-1}), representing a 5.7-fold increase, and produced more than threefold higher total polysaccharide yields. Despite these differences in productivity, polysaccharide preparations IPS and EPS from both species showed comparable antiviral activity. In D. stramonium, both fractions suppressed TMV-induced local lesion formation by up to 98.5%, with inhibition generally increasing with concentration. In contrast, only weak or inconsistent effects were observed in Ch. amaranticolor and N. sylvestris. The results suggest that the antiviral activity of the isolated polysaccharides is primarily mediated by activation of plant defence mechanisms rather than direct virucidal activity. G. lucidum is identified as the more promising candidate for biotechnological production of antiphytoviral polysaccharides.

Key points

• *G. applanatum and G. lucidum produce polysaccharides with antiphytoviral activity*

• *Mycelium and culture liquid are sources of antiviral polysaccharides*

• *G. lucidum yielded over threefold more polysaccharides than G. applanatum*

Introduction

Higher basidiomycetes of the phylum *Basidiomycota* have been used for centuries in traditional medicine for the treatment and prevention of various diseases (Vieira Gomes et al. 2019; Chugh et al. 2022; Łysakowska et al. 2023; Zade et al. 2025). Species of the genus *Ganoderma* are among the best-known and most intensively studied medicinal fungi. These macromycetes constitute a rich and renewable source of biologically active compounds with potential therapeutic applications against a wide range of disorders (Ooi 2000; Wu et al. 2024; Aaqilet al. 2026). Although interest in *Ganoderma* species has a long history, systematic investigation of their structurally diverse bioactive metabolites has intensified only during the last few decades (Lindequist et al. 2005; Liu et al. 2024; Sadowska et al. 2026; Aaqilet al. 2026).

Among the various metabolites produced by basidiomycetes, polysaccharides have attracted particular attention due to their broad spectrum of biological activity and potential for large-scale biotechnological

production. Polysaccharides obtained from fungal sources possess several advantageous physicochemical and physiological properties, including high stability, hydrophilicity, biodegradability, and low toxicity. These characteristics make them attractive for applications in biotechnology, pharmacology, agriculture, and environmentally friendly industrial processes (Mohammed et al. 2021; Dedhia et al. 2022; Benalaya 2024). In addition, due to structural diversity and functional properties, fungal polysaccharides also increasingly used in nanotechnology, such as microencapsulation, surface modification, and targeted drug delivery (Gopinath et al. 2018; Prasher et al. 2021; Beach et al. 2024; Benalaya 2024; Ramadan et al. 2025).

Microbial and fungal polysaccharides are especially attractive for industrial production. Submerged cultivation provides a controlled environment that ensures high purity of the target fractions, enables precise regulation of the fermentation parameters, and allows simultaneous recovery of multiple valuable metabolites from a single culture. While the antiviral properties of natural sulfated and water-soluble polysaccharides are well documented in medical research (Claus-Desbonnet et al. 2022; Liyanage et al. 2023; Sharma et al. 2024; Wang et al. 2024), these polymers have more recently gained attention as environmentally safe agents for crop protection and sustainable agriculture.

Only a limited number of biological compounds are known to effectively suppress plant virus infections. Nevertheless, microbial glycopolymers and exopolysaccharides have demonstrated high inhibitory activity against several economically important phytopathogenic viruses, including tobacco mosaic virus (TMV) and cucumber mosaic virus (Sano 1995; Ipper et al. 2008; Kiprianova et al. 2017). Their protective effects are generally achieved by two main mechanisms: direct virulicidal action (inhibition of viral adsorption, replication, or cell-to-cell movement) or activation of endogenous plant defence responses, such as systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Aoki et al. 1993; Wiesel et al. 2014; Carezzano et al. 2023).

Previous studies from our laboratory have shown that extracellular polysaccharides isolated from the culture liquids of *Tremella mesenterica*, *Ganoderma applanatum*, and *Ganoderma lucidum* possess pronounced antiphytoviral activity and effectively induce resistance to viral infections in plants (Kovalenko et al. 2008, 2009; Kovalenko and Wasser 2018). Similar activity was later demonstrated for structural cell-wall glucans isolated from *Ganoderma adspersum* (Kovalenko et al. 2010). In a more recent study, we found that structural polysaccharides from *G. applanatum* and *G. lucidum* reduced TMV infectivity in leaves of the hypersensitive indicator plant *D. stramonium* (Kovalenko et al. 2024). However, the yields of these fractions were relatively low due to limitations in inoculum preparation and non-optimized cultivation conditions. Moreover, no direct comparison has yet been made between the antiphytoviral activity of extracellular (EPS) and structural (intracellular, IPS) polysaccharides from these two *Ganoderma* species.

The aim of the present study was to compare *G. applanatum* and *G. lucidum* as producers of structural and extracellular polysaccharides with antiphytoviral activity under optimized submerged cultivation conditions. We also assessed the antiviral activity of the isolated fractions against TMV in indicator

plants that differ in their capacity to localize viral infection. These results may be useful for the future development of fungal polysaccharide-based antiviral formulations and plant defence modulators for sustainable crop protection.

Materials and Methods

Virus and Plants

The U1 strain of tobacco mosaic virus (TMV) was used as a model virus. Viral inoculum was prepared from systemically TMV-infected leaves of *Nicotiana tabacum* L. cv. Immune 580 and purified by differential centrifugation according to Love et al. (2015). Antiviral activity was assessed using three TMV indicator plants that differ in their capacities to localize viral infection: *Datura stramonium* L. (high), *Chenopodium amaranticolor* Costa et Reyn. (moderate), and *Nicotiana sylvestris* L. (low).

Plants were grown in a greenhouse under natural light and temperature conditions with drip irrigation. Experiments were conducted on plants at the 4–6 true-leaf stage before the onset of flowering. During the winter period, plants were maintained in a climate chamber under controlled lighting conditions (10,000 lux, 16-hour photoperiod). In all experiments, 8–10 leaves were used per treatment.

Mechanical inoculation was performed by the half-leaf method. The left half of each leaf was treated with the test preparation, while the right half served as the control. TMV was applied at a concentration of 5–10 μg mL^{-1}.

Cultivation of fungal strains

Two basidiomycete strains, *Ganoderma applanatum* 920 and *Ganoderma lucidum* 1900, were selected based on their previously reported production of antiphytoviral exometabolites (Kovalenko et al. 2008). Both strains were obtained from the culture collection of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, and kindly provided by Prof. N.A. Bisko.

The fungi were cultivated in glucose-peptone-yeast extract (GPY) liquid medium containing (g L^{--1}): glucose – 20; peptone – 3; yeast extract – 3; KH_2PO_4 – 1; K_2HPO_4 – 1; $MgSO_4 \times 7H_2O$ – 0.25. The initial pH was adjusted to 6.5. Inoculum cultures were maintained on the same medium solidified with 20 g L^{-1} agar (Difco, Serva, USA). All medium components were of analytical grade with purity above 99%) (Alsi Ltd., Ukraine). The media were sterilized at 121°C (50.66 kPa) for 60 min.

Cultivation was performed in two stages. First, the strains were grown on the solid GPY medium for 7 days at 28° C. The resulting mycelium was then homogenized in a sterile Waring blender and used as seed culture. Aliquots of the homogenate were transferred into 250-ml Erlenmeyer flasks containing 150 ml of liquid GPY medium. Three independent flasks were prepared for each strain. The cultures were

incubated on an orbital shaker (Steinberg system, Expondo GmbH, Germany) at 190 rpm and 28° C for 14 days.

During cultivation, beginning on day 3, 5-mL samples of culture liquid were collected every second day to monitor mycelial dry weight, exopolymer concentration, and medium pH. The mycelium was separated by centrifugation at 7,000 × g for 10 min. Exopolymers were precipitated from the supernatant with three volumes of 96% ethanol at 2–4°C and recovered by centrifugation at 7,000 × g, 10 min. Both mycelial biomass and ethanol precipitates were lyophilized using a LyoQuest plus Telstar freeze-dryer (Azbil Telstar Technologies, Spain) and weighed on an Axis electronic balance (Axis Sp. z.o.o., Poland).

On day 7, the volume removed during sampling was replaced with an equivalent volume of tenfold-concentrated sterile GPY medium. At the end of cultivation, the mycelium was harvested, washed with physiological saline, lyophilized, and stored at 2–4°C. Mycelial morphology was examined during cultivation and after homogenization using a Leitz HM-LUX light microscope (Ernst Leitz Wetzlar GmbH, Germany).

Isolation and purification of polysaccharides

Structural (intracellular) polysaccharides (IPS)

Structural polysaccharides were extracted from lyophilized mycelium using a modified protocol previously developed for *Ganoderma* species (Kovalenko et al. 2010), based on the method for isolation of glycans, specifically β-(1→3)-β-(1→6)-glucan (ganoderan) from basidiomycete fruiting bodies (Misaki et al. 1988). Three fractions were obtained through sequential extractions: hot water (IPS I), acid (IPS II), and alkali (IPS III) (Fig. 3).

In the present study, 0.1 M HCl was used for acidic extraction instead of sulfuric acid. Alkaline extraction was performed with 1 M NaOH containing 0.05% $NaBH_4$ (1:5, w/v). The suspension was heated at 100°C for 5 h. The extraction was repeated twice, and the filtrates were combined and adjusted to pH 4.0 with concentrated HCl. All extracts (IPS I – IPS III) were dialyzed against distilled water, concentrated fivefold under reduced pressure, and deproteinized using a chloroform-isoamyl alcohol mixture (10:1, v/v). The aqueous phase was recovered by centrifugation (7,000 × g, 20 min), dialyzed again, and lyophilized.

Extracellular polysaccharides (EPS)

Extracellular polysaccharides were isolated from the ethanol precipitates obtained from culture liquid and purified using the same deproteinization and dialysis procedure as for IPS (Fig. 3). Yields of IPS and EPS were determined gravimetrically (Skoog et al. 2013). Biomass and EPS production were expressed as grams per liter of culture medium (g L^{-1}). Yields of individual IPS fractions were expressed as milligrams per gram of dry mycelial biomass (mg g^{-1} dry mycelium).

Plant inoculation and antiviral activity assays

To evaluate antiviral activity, aqueous solutions of polysaccharides (1–1000 μg mL^{-1}) were mixed with an equal a volume of purified TMV suspension (10 μg mL^{-1}). After incubation for 30 min at room temperature, the mixtures were mechanically inoculated onto the left halves of the indicator plant leaves. The right halves were inoculated with TMV diluted in 0.1 M phosphate buffer (pH 7.2) as controls. The leaves were rinsed with distilled water 10 min after inoculation.

At 5–7 days post-inoculation the total numbers of local lesions produced on the test and control half leaves were counted. Antiviral activity was expressed as the percentage inhibition of lesion formation and calculated using the formula:

$$\mathrm{I}\ (\%) = \frac{C-E}{C} \times 100,$$

where C is the mean number of lesions on the control half-leaves and E is the mean number on the treated half-leaves. Each treatment was evaluated in three independent experiments.

Statistical analysis

The quantitative parameters of biomass production, polysaccharide yields, and antiviral activity data were evaluated using parametric criteria (Petrovska et al. 2022). Data in the tables and diagrams are presented as means ± standard deviations (SD). Significance of differences between means was determined by Student's t-test and denoted as follows: $p \leq 0.001$; $0.001 < p \leq 0.01$; $p \leq 0.05$; ns - not significant.

Results

Growth characteristics and polysaccharide production of Ganoderma species under submerged cultivation

G. applanatum and *G. lucidum* were cultivated in GPY medium containing 20 g L^{-1} glucose for 14 days under submerged conditions. The main stages of cultivation and biomass recovery are presented in Fig. 1. The two species differed in colony morphology on solid medium and in the macroscopic appearance of submerged cultures. During cultivation, *G. applanatum* formed a dark-brown culture broth, whereas the broth of *G. lucidum* remained lighter in colour. The harvested mycelium of *G. lucidum* formed large, dense aggregates, while *G. applanatum* mycelium was less abundant and more fragmented. Dynamics of mycelial biomass accumulation, extracellular polysaccharide production, and medium pH during cultivation are shown in Fig. 2.

In both *Ganoderma* species, biomass and exopolymer production increased throughout the cultivation period, but *G. lucidum* produced accumulated substantially more mycelial biomass than *G. applanatum*. The accumulation of extracellular metabolites was accompanied by a gradual decrease in medium pH.

The decrease in medium pH was more pronounced in *G. lucidum* cultures (from 6.5 to 4.0) than in *G. applanatum* (from 6.5 to 5.2) by day 14.

By the end of cultivation, mycelial biomass reached 21.5 g L-1 in *G. lucidum* and 3.8 g L-1 in *G. applanatum*. The accumulation of extracellular polymers recovered by ethanol precipitation followed a different pattern. During the first half of cultivation, exopolymer production was higher in *G. applanatum*. However, the final concentrations were comparable and reached 2.10 g L^{-1} and 2.64 g L^{-1} for *G. applanatum* and *G. lucidum*, respectively (Fig. 2). Following supplementation of the cultures with concentrated medium on day 7, biomass accumulation and exopolymer production increased in both species. This response was more pronounced in *G. lucidum*, which showed a rapid increase in both parameters during the second half of cultivation.

A workflow for the isolation and purification of structural (intracellular) and extracellular polysaccharides from the cultivated biomass is shown in Fig. 3. The procedure included sequential hot-water (IPS I), acid (0.1 M HCl, IPS II), and alkaline (0.1 M NaOH, IPS III) extraction of the mycelial biomass, combined with ethanol precipitation of EPS from the culture liquid. This approach enabled the isolation of three structural polysaccharide fractions (IPS I – IPS III) and purified extracellular polysaccharides.

The yields of IPS and EPS from both fungal species are summarized in Table 1. The highest recovery of IPS was achieved during alkaline extraction. Fraction IPS III accounted for 22.4% of the dry biomass in *G. applanatum* and 27.1% in *G. lucidum*, whereas the yields of the acid-soluble fraction IPS II were similar in both species (5.6–5.7%). Water-soluble polysaccharides represented only a minor proportion of the total polysaccharide content. The total IPS yields reached 28.6% in *G. applanatum* and 34.8% in *G. lucidum*, while the recovery of purified EPS from crude ethanol precipitates was 13.8% and 39.8%, respectively.

Volumetric productivity was markedly higher in *G. lucidum*. Structural polysaccharide production reached 11.33 g L^{-1} in *G. lucidum* compared with 2.04 g L^{-1} in *G. applanatum*. The corresponding EPS yields were 8.93 g L^{-1} and 0.27 g L^{-1}, respectively. As a result, the cumulative yield of purified polysaccharides was 20.26 g L^{-1} (3.04 g per batch) for *G. lucidum* and only 2.31 g L^{-1} (0.34 g per batch) for *G. applanatum*. Despite these pronounced differences in productivity, both species produced sufficient quantities of IPS and EPS for biological evaluation.

Table 1
Recovery of structural (IPS) and extracellular (EPS) polysaccharides from submerged cultures of *G. applanatum* and *G. lucidum*

Producer	Fraction / Sample	Dry mycelium (g)	Recovered IPS (g)	Yield (% DW)	CEP (g)	Recovered EPS (g)	Yield (%)
G. applanatum	IPS I (water-soluble)	1.07	0.006	0.6	0.29	0.04	13.8
	IPS II (acid-soluble)		0.060	5.6			
	IPS III (alkali-soluble)		0.240	22.4			
	Total IPS		**0.306**	**28.6**			
G. lucidum	IPS I (water-soluble)	4.88	0.100	2.1	3.37	1.34	39.8
	IPS II (acid-soluble)		0.280	5.7			
	IPS III (alkali-soluble)		1.320	27.1			
	Total IPS		**1.700**	**34.8**			

DW – dry mycelial weight; CEP – crude ethanol precipitate obtained from culture liquid before purification. Yield (% DW) was calculated relative to dry mycelial biomass. EPS yield (%) was calculated relative to CEP mass. Total recovered polysaccharides (IPS + EPS) were 0.346 g for *G. applanatum* and 3.040 g for *G. lucidum*.

Antiviral activity of isolated polysaccharides against TMV

The antiviral activity of the isolated preparations was evaluated against TMV using three indicator plants differing in their capacity to localize viral infection: *D. stramonium* L. (high), *Ch. amaranticolor* Costa et Reyn (moderate), and *N. silvestris* L. (low). Representative symptoms induced by TMV in these plants are shown in Fig. 4. The severity and localization of necrotic lesions increased in the order *D. stramonium* - *Ch. amaranticolor* - *N. sylvestris*.

Both structural polysaccharides (fractions IPS I, IPS II, IPS III) isolated from mycelium (Table 2) and extracellular polysaccharides recovered from culture liquid (Table 3) reduced TMV infectivity in *D. stramonium.* In *D. stramonium*, all polysaccharide fractions exhibited strong, concentration-dependent antiviral activity. Inhibition of local lesion formation ranged from 20.5 to 98.5%, depending on the fungal

species, polysaccharide fraction, and concentration. The strongest effects were observed at 1000 µg mL^{-1}, where all IPS fractions suppressed lesion formation by 89.1–98.0% (Table 2), while EPS preparations reduced lesion numbers by 97.2–98.5% (Table 3). Significant inhibition was also recorded at lower concentrations (1–100 µg mL^{-1}), particularly for IPS I and IPS II. In contrast, the effects in *C. amaranticolor* and *N. sylvestris* were much weaker and often statistically non-significant. Several preparations even stimulated lesion formation in these plants. In *Ch. amaranticolor*, the highest inhibition was recorded for the IPS II fraction of *G. lucidum* at 1000 µg mL^{-1} (36.2 ± 8.0%; $p \leq 0.01$). A comparable effect was observed for EPS from the same species and concentration (36.2 ± 7.5%; $p \leq 0.01$). In *N. sylvestris*, inhibition rarely exceeded 20%. No substantial differences in antiviral efficacy were observed between the two fungal species or between IPS and EPS fractions. The strongest and most consistent inhibition occurred in *D. stramonium*, whereas the effects in *C. amaranticolor* and *N. sylvestris* were considerably weaker.

Table 2
Inhibition of TMV local lesion formation by structural polysaccharides isolated from *Ganoderma* spp. in indicator plants with different levels of virus localization

Producer	**Fraction**	**Concentration (μg mL⁻¹)**	***D. stramonium* (%)**	***Ch. amaranticolor* (%)**	***N. sylvestris* (%)**
G. applanatum	IPS I	1	52.7 ± 6.7^{c}	–	–
		10	67.6 ± 8.5 ^{c}	2.2 ± 1.2 ns	1.8 ± 0.2 ns
		100	88.9 ± 6.2	6.3 ± 9.1 ns	4.7 ± 1.2 ns
		1000	97.3 ± 3.6^{a}	20.7 ± 7.4^{c}	6.3 ± 1.5 ns
G. applanatum	IPS II	1	39.7 ± 4.5^{c}	–	–
		10	63.4 ± 4.1	6.3 ± 5.9 ns	stimulation
		100	78.4 ± 3.0^{a}	0.9 ± 0.1 ns	6.2 ± 0.1 ns
		1000	93.8 ± 1.8^{a}	13.8 ± 3.1^{c}	21.5 ± 0.1^{a}
G. applanatum	IPS III	1	28.5 ± 7.6^{c}	–	–
		10	64.1 ± 5.3	5.2 ± 1.1 ns	stimulation
		100	74.3 ± 4.9^{a}	11.0 ± 6.0 ns	stimulation
		1000	91.9 ± 1.7^{a}	Stimulation	6.5 ± 2.1 ns
G. lucidum	IPS I	1	49.2 ± 5.4	–	–
		10	57.9 ± 6.8	2.8 ± 2.3 ns	3.0 ± 0.2 ns
		100	80.4 ± 5.3	Stimulation	stimulation
		1000	98.0 ± 1.5^{a}	Stimulation	11.4 ± 1.2^{c}
G. lucidum	IPS II	1	50.4 ± 9.7^{c}	–	–
		10	68.8 ± 6.8	10.5 ± 1.2^{c}	8.4 ± 1.3^{c}
		100	85.6 ± 3.3^{a}	26.8 ± 5.1^{c}	16.5 ± 1.0^{c}
		1000	93.7 ± 1.8	36.2 ± 8.0	15.8 ± 1.6^{c}
G. lucidum	IPS III	1	39.1 ± 8.1 ns	–	–
		10	62.8 ± 6.0	Stimulation	stimulation

Producer	Fraction	Concentration (μg mL⁻¹)	*D. stramonium* (%)	*Ch. amaranticolor* (%)	*N. sylvestris* (%)
		100	75.2 ± 4.2	24.5 ± 0.1[c]	4.0 ± 1.1 ns
		1000	89.1 ± 2.2[a]	15.8 ± 6.3 ns	10.7 ± 2.1[c]

Values represent inhibition (%) of TMV local lesion formation relative to the corresponding control. Data are presented as mean ± SD (n = 3). p ≤ 0.001; 0.001 < p ≤ 0.01; p ≤ 0.05; ns - not significant.

Table 3
Inhibition of TMV local lesion formation by extracellular polysaccharides isolated from *Ganoderma* spp. in indicator plants with different levels of virus localization

Producer	Concentration (μg mL⁻¹)	*D. stramonium* (%)	*Ch. amaranticolor* (%)	*N. sylvestris* (%)
G. applanatum	1	20.5 ± 7.8[c]	–	–
	10	42.9 ± 7.4[c]	stimulation	stimulation
	100	63.7 ± 5.1[b]	stimulation	8.0 ± 7.4 ns
	1000	97.2 ± 8.3[a]	6.6 ± 5.7 ns	stimulation
G. lucidum	1	26.3 ± 8.9[c]	–	–
	10	38.0 ± 7.4[c]	7.2 ± 4.2 ns	3.1 ± 8.3 ns
	100	79.2 ± 7.6[a]	10.0 ± 5.6[a]	9.1 ± 4.7 ns
	1000	98.5 ± 5.1[a]	36.2 ± 7.5[b]	22.2 ± 6.4[c]

Values represent inhibition (%) of TMV local lesion formation relative to the corresponding control. Data are presented as mean ± SD (n = 3). p ≤ 0.001; 0.001 < p ≤ 0.01; p ≤ 0.05; ns - not significant.

Discussion

Basidiomycetes are important producers of structurally diverse polysaccharides with applications in pharmacy, nutraceuticals, and agriculture (Mizuno 1999; Wasser 2002; Kovalenko et al. 2008, 2010; Kovalenko and Wasser 2018; Vieira Gomes et al. 2019; Kumla et al. 2025). The development of submerged cultivation techniques has transformed fungal mycelium into an accessible and renewable source of bioactive compounds that are often difficult to obtain in sufficient quantities from natural fruiting bodies. In addition, submerged cultivation provides a controlled production system that allows targeted regulation of metabolite synthesis through modification of medium composition and cultivation

conditions, while a dispersed mycelial morphology facilitates subsequent extraction and purification of both intracellular and extracellular metabolites.

In our earlier study, the yields of polysaccharide fractions obtained from of *G. applanatum* and *G. lucidum* were limited by relatively low biomass accumulation caused by suboptimal inoculum preparation and cultivation conditions (Kovalenko et al. 2024). In the present study, these constraints were overcome by homogenizing the agar-grown seed culture, extending the cultivation period to 14 days, and replenishing the medium during active growth (on day 7). These modifications led to markedly higher biomass accumulation and exopolymer production (Fig. 2) and, consequently, substantially improved polysaccharide recovery (Table 1). Under the chosen conditions, *G. lucidum* grew more efficiently than *G. applanatum*. At the end of cultivation, its mycelial biomass reached 21.5 g L^{-1} $^{-1}$ compared with only 3.8 g L^{-1} for *G. applanatum*. Although the exopolymer synthesis was initially more intensive in *G. applanatum*, the final volumetric yields were similar. The pH of the culture medium dropped from 6.5 to 4.0 in *G. lucidum* and to 5.2 in *G. applanatum* cultures. *G. lucidum* also responded more strongly to medium replenishment on day 7, which was reflected in renewed biomass and exopolymer accumulation. Together, these observations point to significant physiological differences between the two species during submerged growth. Similar effects of inoculum preparation and cultivation conditions on biomass formation and polysaccharide production have been reported previously for submerged cultures of *Ganoderma* spp. and other medicinal basidiomycetes (Papinutti 2010; Yuan et al. 2012; Liu and Zhang 2019; Supramani et al. 2019, 2023). The higher biomass accumulation observed in *G. lucidum* was accompanied by a stronger acidification of the medium pH and a more pronounced response to nutrient supplementation, indicating more intensive substrate utilization during cultivation and metabolic activity. Similar changes have been described for submerged *Ganoderma* cultures, where active glucose consumption correlates with medium acidification, biomass formation, and polysaccharide synthesis (Yang and Liau 1998; Fang and Zhong 2002; Babitskaya et al. 2005).

Extraction procedures have a major impact on the yield, structural integrity, and biological properties of fungal polysaccharides (Liu and Cheung 2019; Murphy et al. 2020). To enhance recovery of antiphytoviral fractions, we developed an optimized sequential extraction protocol (Fig. 3). Unlike our earlier methods (Kovalenko et al. 2008, 2010; Kovalenko and Wasser 2018), hot-water, acid, and alkaline extractions were performed consecutively on the same mycelial biomass. We used 0.1 M HCl instead of sulfuric acid for acidic extraction and 1 M NaOH instead of supplemented with $NaBH_4$ for alkaline extraction. This modification increased the recovery of acid- and alkali-soluble fractions and reduced the risk of alkaline degradation during extraction.

The total yield of intracellular polysaccharides recovered from *G. lucidum* reached 11.33 g L^{-1}, and extracellular polysaccharide production reached 8.93 g L^{-1}. These values are higher than most reported previously yields for submerged cultures of *G. lucidum*. EPS yields in the literature typically range from 0.4 to 5.5 g L^{-1} depending on strain, medium composition and cultivation conditions (Yuan et al. 2012; Abdullah et al. 2020; Supramani et al. 2023). Higher values have been achieved under specifically optimized conditions, with some reports reaching up to approximately 15 g L^{-1} (Papinutti 2010). Yields

of IPS most often fall within the range of 1.5–3.5 g L^{-1} (Abdullah et al. 2020; Supramani et al. 2023; Kumla et al. 2025). Although direct comparison across studies are difficult due to differences in strains, media, extraction and quantification methods, the cultivation and sequential extraction strategy employed in this study enables efficient simultaneous recovery of both IPS and EPS within a single production cycle.

The modifications introduced in this study resulted in substantially higher yields of structural and extracellular polysaccharides compared with our previous experiments with the same fungal strains (Kovalenko et al. 2024), particularly in the acid- and alkali-soluble fractions. The predominance of the alkali-soluble fraction in both species is consistent with the high content of alkali-extractable β-glucans of *Ganoderma* cell walls (Misaki et al. 1988; Liu and Cheung 2019). From a biotechnological perspective, the proposed workflow allows recovery of IPS and EPS from one cultivation cycle, thereby increasing the overall efficiency of biomass and culture liquid utilization.

The antiviral activity of the obtained preparations was evaluated using three indicator plants differing in their ability to localize TMV infection. Consistent with our earlier results on extracellular polysaccharides isolated from *Ganoderma* spp. and *Tremella mesenterica* (Kovalenko et al. 2008; 2009; Kovalenko and Wasser 2018), both IPS and EPS suppressed TMV infection most effectively in *D. stramonium*. Inhibition of local lesion formation ranged from 28 to 98% for structural polysaccharides and from 20 to 99% for extracellular polysaccharides, depending on fraction and concentration. In contrast, the same preparations exhibited much weaker activity in *Ch. amaranticolor* and *N. sylvestris*, species characterized by less efficient virus localization.

No substantial differences in antiviral efficacy were found between *G. applanatum* and *G. lucidum* or between the IPS and EPS fractions. The strongest and most consistent activity was observed in *D. stramonium*, which exhibits a well-developed hypersensitive response, whereas only limited effects were detected in *C. amaranticolor* and *N. sylvestris*, species characterized by weaker localization of viral infection. These results indicate that the antiviral effect of the polysaccharides depends primarily on the activation of host plant defence mechanisms rather than on direct virucidal activity or the fungal source of the polysaccharides. This interpretation is supported by the earlier observations for polysaccharides from *G. adspersum* (Kovalenko et al. 2010) and by reports describing fungal β-glucans as effective elicitors of plant immunity (Aoki et al. 1993; Wiesel et al. 2014; Carezzano et al. 2023; Zhu et al. 2024). Recognition of such polysaccharides by plant cells can trigger reactive oxygen species production, activate defence signalling pathways, induce the expression of pathogenesis-related genes, and promote the development of local and systemic resistance responses (Goldman et al. 2021; Zhu et al. 2024).

Despite large differences in biomass accumulation and polysaccharide productivity, preparations from *G. applanatum* and *G. lucidum* displayed comparable antiphytoviral activity. Thus, under the conditions examined here, biological efficacy was not directly related to the quantity of polysaccharides produced. From a biotechnological perspective, however, *G. lucidum* proved to be more productive species and clearly outperformed *G. applanatum* in the yields of polysaccharides. At the same time, polysaccharide

fractions from *G. applanatum* retained comparable biological activity and therefore remain a valuable potential source of antiphytoviral glycans. Further optimization of cultivation and extraction conditions may improve polysaccharide recovery and overall productivity for both species.

Conclusions

Standardized submerged cultivation conditions and a sequential extraction protocol were developed for the efficient recovery of intracellular and extracellular polysaccharides from *Ganoderma applanatum* and *G. lucidum*. The optimized scheme not only substantially increased polysaccharide yields compared with previous studies, but also preserved high antiphytoviral activity of the isolated fractions. It clearly demonstrated the advantage of *G. lucidum* over *G. applanatum* as a producer of antiphytoviral glycans, especially intracellular fractions. Polysaccharides from both species showed high activity against TMV in *Datura stramonium*, a host with a strong hypersensitive response, but exhibited only weak or inconsistent effects in *Chenopodium amaranticolor* and *Nicotiana sylvestris*, species with limited or absent virus localization capacity. These data indicate that the polysaccharides act primarily as elicitors of plant defence mechanisms rather than as direct virucidal agents. The developed cultivation and isolation scheme provides a useful basis for further studies of the structure–activity relationships of these fungal polysaccharides and their application in plant protection technologies. The developed cultivation and isolation scheme provides a useful basis for further studies of the structure–activity relationships of these fungal polysaccharides and their application in plant protection technologies.

Declarations

Author contributions All authors contributed to the study conception, design, experiment and data collection. AK and OK conceived and designed the experimental design. OK and TB performed the experiments. AK provided new analytical tools. OK and Yu Sh analyzed and formatted the data in tables and figures. OK wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical approval. This article does not contain any studies involving human participants or animals performed by any of the authors.

Competing interests The authors declare no competing interests.

References

1. Abdullah NR, Sharif F, Azizan NH, Hafidz IFM, Supramani S, Usuldin SRA, Ahmad R, Wan-Mohtar WAAQI (2020) Pellet diameter of Ganoderma lucidum in a repeated-batch fermentation for the trio total production of biomass-exopolysaccharide-endopolysaccharide and its anti-oral cancer beta-glucan response. AIMS Microbiol. 6(4): 379-400. https://doi.org/10.3934/microbiol.2020023
2. Aaqil M, Zhang F, Zheng J, Kamil M, Guo Y, Zhao C, Tian Y (2026) Fungal Polysaccharides as Next-Generation Bioactive: Advances in Structure–Activity Mapping, Mode of Action, and Biotechnological Horizons. Food Frontiers 7(3): e70238. https://doi.org/10.1002/fft2.70238
3. Aoki M, Tan M, Fukushima A, Hieda T, Kubo S, Takabayashi M, Ono K, Mikami Y (1993) Antiviral Substances with Systemic Effects Produced by Basidiomycetes such as Fomes fomentarius. Biosci Biotechnol Biochem. 57(2): 278-82. https://doi.org/10.1271/bbb.57.278
4. Beach MA, Nayanathara U, Gao Y, Zhang C, Xiong Y, Wang Y, Such GK (2024) Polymeric Nanoparticles for Drug Delivery. Chem Rev. 124 (9): 5505-5616. https://doi.org/10.1021/acs.chemrev.3c00705
5. Benalaya I, Alves G, Lopes J, Silva LR (2024) A Review of Natural Polysaccharides: Sources, Characteristics, Properties, Food, and Pharmaceutical Applications. Int J Mol Sci. 25(2): 1322. https://doi.org/10.3390/ijms25021322
6. Carezzano ME, Alvarez Strazzi FB, Pérez V, Bogino P, Giordano W (2023) Exopolysaccharides Synthesized by Rhizospheric Bacteria: A Review Focused on Their Roles in Protecting Plants against Stress. Appl Microbiol 3(4): 1249-1261. https://doi.org/10.3390/applmicrobiol3040086
7. Chugh RM, Mittal P, Mp N, Arora T, Bhattacharya T, Chopra H, Cavalu S, Gautam RK (2022) Fungal Mushrooms: A Natural Compound With Therapeutic Applications. Front Pharmacol. 13: 13: 925387. https://doi.org/10.3389/fphar.2022.925387.
8. Claus-Desbonnet H, Nikly E, Nalbantova V, Karcheva-Bahchevanska D, Ivanova S, Pierre G, Benbassat N, Katsarov P, Michaud P, Lukova P, Delattre C (2022) Polysaccharides and Their Derivatives as Potential Antiviral Molecules. Viruses. 14(2): 426. https://doi.org/10.3390/v14020426
9. Dedhia N, Marathe SJ, Singhal RS (2022) Food polysaccharides: A review on emerging microbial sources, bioactivities, nanoformulations and safety considerations. Carbohydr Polym. 287: 119355. https://doi.org/10.1016/j.carbpol.2022.119355.
10. Gopinath V, Saravanan S, Al-Maleki AR, Ramesh M, Vadivelu J. (2018) A review of natural polysaccharides for drug delivery applications: Special focus on cellulose, starch and glycogen. Biomed Pharmacother. 107: 96-108. https://doi.org/10.1016/j.biopha.2018.07.136.
11. Goldman GH, Delneste Y, Papon N (2021) Fungal Polysaccharides Promote Protective Immunity. Trends Microbiol. May;29(5):379-381. https://doi.org/10.1016/j.tim.2021.02.004.

12. Ipper NS, Cho S, Lee SH, Cho JM, Hur JH, Lim CK (2008) Antiviral activity of the exopolysaccharide produced by Serratia sp. strain Gsm01 against Cucumber mosaic virus. J Microbiol Biotechnol. 18(1): 67-73.
13. Kiprianova EA, Safronova LA, Prosyanov AO (2017) Antimicrobial, entomopathogenic and antiviral activity of gaupsin biopreparation created on the basis of Pseudomonas chlororaphis strains. Biotechnologia Acta 10(1): 7–16. https://doi.org/10.15407/biotech10.01.007
14. Kovalenko O, Kyrychenko A, Shchukin Y, Bondarenko T (2025) Comparative activity of Ganoderma applanatum and Ganoderma lucidum as producers of antiviral polysaccharides. In: One world – one health: proceedings of the II international scientific and practical conference. Institute of Climate-Smart Agriculture NAAS, Odesa, pp 227–230
15. Kovalenko OG, Polishchuk OM, Krupodorova TA, Bisko NA, Buchalo AS (2008) Screening of metabolites produced by strains of Ganoderma lucidum [Curt.:Fr] P.Karst and Ganoderma applanatum [Pirs.:Waller] Pat. for their activity against tobacco mosaic virus. Bulletin of Taras Shevchenko National University of Kyiv. Biology. 51: 32–34.
16. Kovalenko OG, Polishchuk OM, Wasser SP (2010) Glycans of higher basidiomycete Ganoderma adspersum (Schulzer) Donk: preparation and antiphytoviral activity. Biotechnology. 3: 83–91.
17. Kovalenko OG, Polishchuk ON, Wasser SP (2009) Virus Resistance Induced by Glucuronoxylomannan Isolated from Submerged Cultivated Yeast-like Cell Biomass of Medicinal Yellow Brain Mushroom Tremella mesenterica Ritz.: Fr. (Heterobasidiomycetes) in Hypersensitive Host Plants. Int J. Med. Mushrooms. 11: 199–205. https://doi.org/10.1615/IntJMedMushr.v11.i2.90
18. Kovalenko OG, Wasser SP (2018) Glycans of higher basidiomycetes mushrooms with antiphytoviral properties: isolation, characterization, and biological activity. In: Fungi. CRC Press, Boca Raton, pp 161–200. https://doi.org/10.1201/9781315369471-9
19. Kumla J, Thangrongthong S, Kaewnunta A and Suwannarach N (2025) Research advances in fungal polysaccharides: production, extraction, characterization, properties, and their multifaceted applications. Front. Cell. Infect. Microbiol. 15: 1604184. https://doi.org/10.3389/fcimb.2025.1604184
20. Lindequist U, Niedermeyer THJ, Jülich W-D (2005) The Pharmacological Potential of Mushrooms. Evidence-Based Complementary and Alternative Medicine. 2:285–299. https://doi.org/10.1093/ecam/neh107
21. Liu H-M, Cheng J, Wang X-Y, Jiang Y, Ni J, Zhang Y, Wang W (2024) Structure Identification of Ganoderma lucidum Spore Polysaccharides and Their Antitumor Activity In Vivo. Molecules. 29: 2348. https://doi.org/10.3390/molecules29102348
22. Liu SR, Zhang WR (2018) Optimization of submerged culture conditions involving a developed fine powder solid seed for exopolysaccharide production by the medicinal mushroom Ganoderma lucidum. Food Sci Biotechnol. 28(4): 1135-1145. https://doi.org/10.1007/s10068-018-0536-5
23. Liyanage NM, Nagahawatta DP, Jayawardena TU, Sanjeewa KKA, Jayawrdhana HHACK, Kim J-I, Jeon Y-J. (2023) Sulfated Polysaccharides from Seaweeds: A Promising Strategy for Combatting

Viral Diseases - A Review. Marine Drugs. 21(9): 461. https://doi.org/10.3390/md21090461

24. Liu C, Cheung PCK (2019). Structure and immunomodulatory activity of microparticulate mushroom sclerotial β-glucan prepared from Polyporus rhinocerus. Journal of Agricultural and Food Chemistry, 67: 9070-9078. https://doi.org/10.1021/acs.jafc.9b03206
25. Love AJ, Makarov VV, Sinitsyna OV, Shaw J, Yaminsky IV, Kalinina NO and Taliansky ME (2015) A Genetically Modified Tobacco Mosaic Virus that can Produce Gold Nanoparticles from a Metal Salt Precursor. Front. Plant Sci. 6: 984. https://doi.org/10.3389/fpls.2015.00984
26. Łysakowska P, Sobota A, Wirkijowska A (2023) Medicinal Mushrooms: Their Bioactive Components, Nutritional Value and Application in Functional Food Production - A Review. Molecules. 28(14): 5393. https://doi.org/10.3390/molecules28145393
27. Misaki A, Sone Y, Yoshida M, Takeuchi K (1988) Path. 4769363 US, A61K/70; C07H 15/04. Betaglucan
28. Mizuno T (1999) The Extraction and Development of Antitumor-Active Polysaccharides from Medicinal Mushrooms in Japan (Review). Int. J. Med. Mushrooms. 1: 9-29. https://doi.org/10.1615/IntJMedMushrooms.v1.i1.20
29. Mohammed ASA, Naveed M, Jost N (2021) Polysaccharides; Classification, Chemical Properties, and Future Perspective Applications in Fields of Pharmacology and Biological Medicine (A Review of Current Applications and Upcoming Potentialities). J Polym Environ. 29(8): 2359-2371. https://doi.org/10.1007/s10924-021-02052-2.
30. Murphy EJ, Masterson C, Rezoagli E, O'Toole D, Major I, Stack GD. Lynch M, Laffey JG, Rowan NJ (2020) β-Glucan Extracts from the Same Edible Shiitake Mushroom Lentinus edodes Produce Differential in-Vitro Immunomodulatory and Pulmonary Cytoprotective Effects-Implications for Coronavirus Disease (COVID-19) Immunotherapies. Sci. Total Environ. 732: 139330. https://doi.org/10.1016/j.scitotenv.2020.139330
31. Ooi VEC (2000) Medicinally important fungi. In: Van Griensven LJLD (ed) Science and cultivation of edible fungi, vol 1. Balkema, Rotterdam: 41–51
32. Papinutti L (2010) Effects of nutrients, pH and water potential on exopolysaccharides production by a fungal strain belonging to Ganoderma lucidum complex. Bioresour Technol. 101(6): 1941-1946. https://doi.org/10.1016/j.biortech.2009.09.076.
33. Petrovska I, Salyha Y, Vudmaska I (2022) Statistical methods in biological research: educational and methodological manual. Agrarian Science, Kyiv
34. Prasher P, Sharma M, Mehta M, Satija S, Aljabali AA. Tambuwala MM, Anand K, Sharma N, Dureja H, Jha NK, Gupta G, Gulati M, Singh SK, Chellappan DK, Paudel KR, Hansbro PM, Dua K (2021). Current-status and applications of polysaccharides in drug delivery systems. Colloids and Interface Science Communications, 42: 100418. https://doi.org/10.1016/j.colcom.2021.100418
35. Ramadan NE, Youssef FR, Alshishtawy AAK, Elshikh FM, Newir O, Abdelazeem SH, Ma'ruf NK, Shouman H, Ali SS, El-Sheekh MM (2025) Marine algal polysaccharides for drug delivery

applications: A review. Int J Biol Macromol. 295: 139551. https://doi.org/10.1016/j.ijbiomac.2025.139551

36. Sadowska A, Włosek-Pawełas D, Car H (2026) Medicinal Mushrooms and Their Bioactive Compounds: From Traditional Use to Therapeutic Potential. Molecules. 31(10): 1749. https://doi.org/10.3390/molecules31101749
37. Sano Y (1995) Antiviral activity of polysaccharides against infection of tobacco mosaic virus. Macromol. Symp. 99: 239-242. https://doi.org/10.1002/masy.19950990125
38. Sharma A, Shahid A, Banerjee R, Kumar KJ (2024) Emerging insights into the structure-activity relationship of water-soluble polysaccharides in antiviral therapy. Eur J Med Chem Rep. 10: 100122. https://doi.org/10.1016/j.ejmcr.2023.100122
39. Skoog DA, West DM, Holler FJ, Crouch SR (2013) Fundamentals of Analytical Chemistry. Ninth Edition, Cengage Learning, Boston, MA, USA.
40. Supramani S, Ahmad R, Ilham Z, Annuar MSM, Klaus A, Wan-Mohtar WAAQI (2019) Optimisation of biomass, exopolysaccharide and intracellular polysaccharide production from the mycelium of an identified Ganoderma lucidum strain QRS 5120 using response surface methodology. AIMS Microbiol. 5: 19-38. https://doi.org/10.3934/microbiol.2019.1.19
41. Supramani S, Rejab NA, Ilham Z, Ahmad R, Show PL, Ibrahim MF, Wan-Mohtar WAAQI (2023) Performance of Biomass and Exopolysaccharide Production from the Medicinal Mushroom Ganoderma lucidum in a New Fabricated Air-L-Shaped Bioreactor (ALSB). Processes, 11(3), Article 670. https://doi.org/10.3390/pr11030670
42. Vieira Gomes DC, de Alencar MVOB, Dos Reis AC, de Lima RMT, de Oliveira Santos JV, da Mata AMOF, Soares Dias AC, da Costa JS Junior, de Medeiros MDGF, Paz MFCJ, Gayoso E Almendra Ibiapina Moreno LC, Castro E Sousa JM, Islam MT, Melo Cavalcante AAC (2019) Antioxidant, anti-inflammatory and cytotoxic/antitumoral bioactives from the phylum Basidiomycota and their possible mechanisms of action. Biomed Pharmacother. 112: 108643. https://doi.org/10.1016/j.biopha.2019.108643
43. Wang Y, Guo X, Huang C, Shi C, Xiang X (2024) Biomedical potency and mechanisms of marine polysaccharides and oligosaccharides: A review. Int J Biol Macromol. 265 (Pt 1): 131007. https://doi.org/10.1016/j.ijbiomac.2024.131007
44. Wasser S (2002) Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl. Microbiol. Biotechnol. 60:258–274. https://doi.org/10.1007/s00253-002-1076-7
45. Wiesel L, Newton AC, Elliott I, Booty D, Gilroy EM, Birch PRJ, Hein I (2014) Molecular effects of resistance elicitors from biological origin and their potential for crop protection. Front. Plant Sci. 5: 655. https://doi.org/10.3389/fpls.2014.00655
46. Wu S, Zhang S, Peng B, Tana D, Wua M, Weia J, Wanga Y, Luo Hua (2024) Ganoderma lucidum: a comprehensive review of phytochemistry, efficacy, safety and clinical study. Food Science and Human Wellness, 13(2): 568-596. https://doi.org/10.26599/FSHW.2022.9250051

47. Yuan B, Chi X, Zhang R (2012) Optimization of exopolysaccharides production from a novel strain of Ganoderma lucidum CAU5501 in submerged culture. Brazilian Journal of Microbiology 43: 490–497. https://doi.org/10.1590/S1517-83822012000200009
48. Zade S, Upadhyay TK, Rab SO, Sharangi AB, Lakhanpal S, Alabdallah NM, Saeed M (2025) Mushroom-derived bioactive compounds pharmacological properties and cancer targeting: a holistic assessment. Discov Onc 16: 654. https://doi.org/10.1007/s12672-025-02371-z
49. Zhu F, Cao MY, Zhang QP, Mohan R, Schar J, Mitchell M, Chen H, Liu F, Wang D, Fu ZQ (2024) Join the green team: Inducers of plant immunity in the plant disease sustainable control toolbox. J Adv Res. 57: 15-42. https://doi.org/10.1016/j.jare.2023.04.016.

Figures

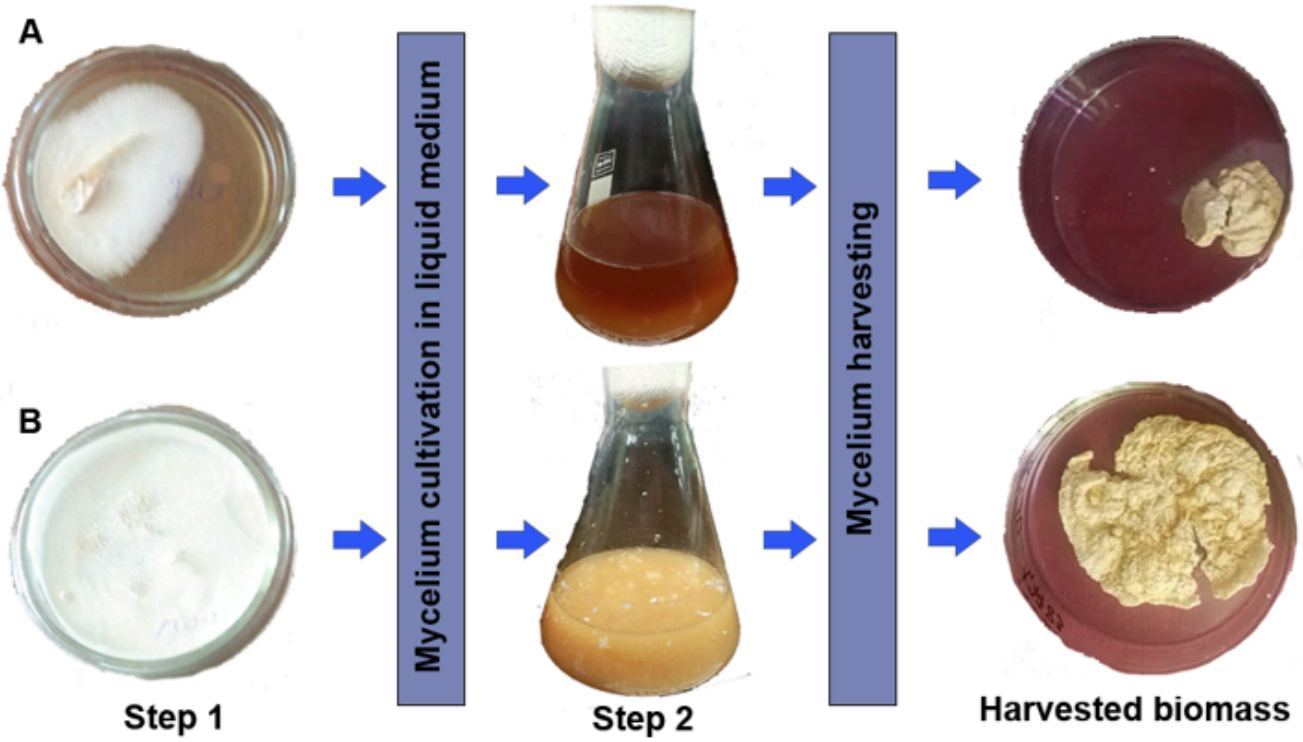


Figure 1

Main stages of submerged cultivation and biomass recovery of *G. applanatum* (A) and *G. lucidum* (B). Step 1, growth on solid GPY medium; Step 2, submerged cultivation in liquid GPY medium for 14 days; Step 3: harvested mycelial biomass

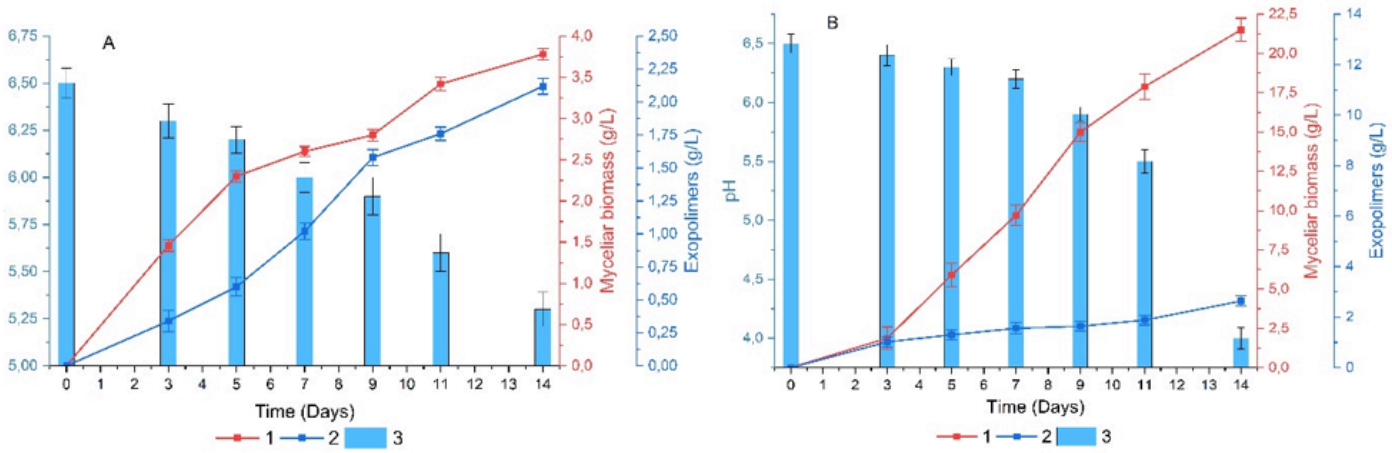


Figure 2

Dynamics of mycelial biomass accumulation (1), extracellular polysaccharide production (2), and pH changes (3) during submerged cultivation of *G. applanatum* (A) and *G. lucidum* (B). Data are presented as mean ± SD (n = 3).

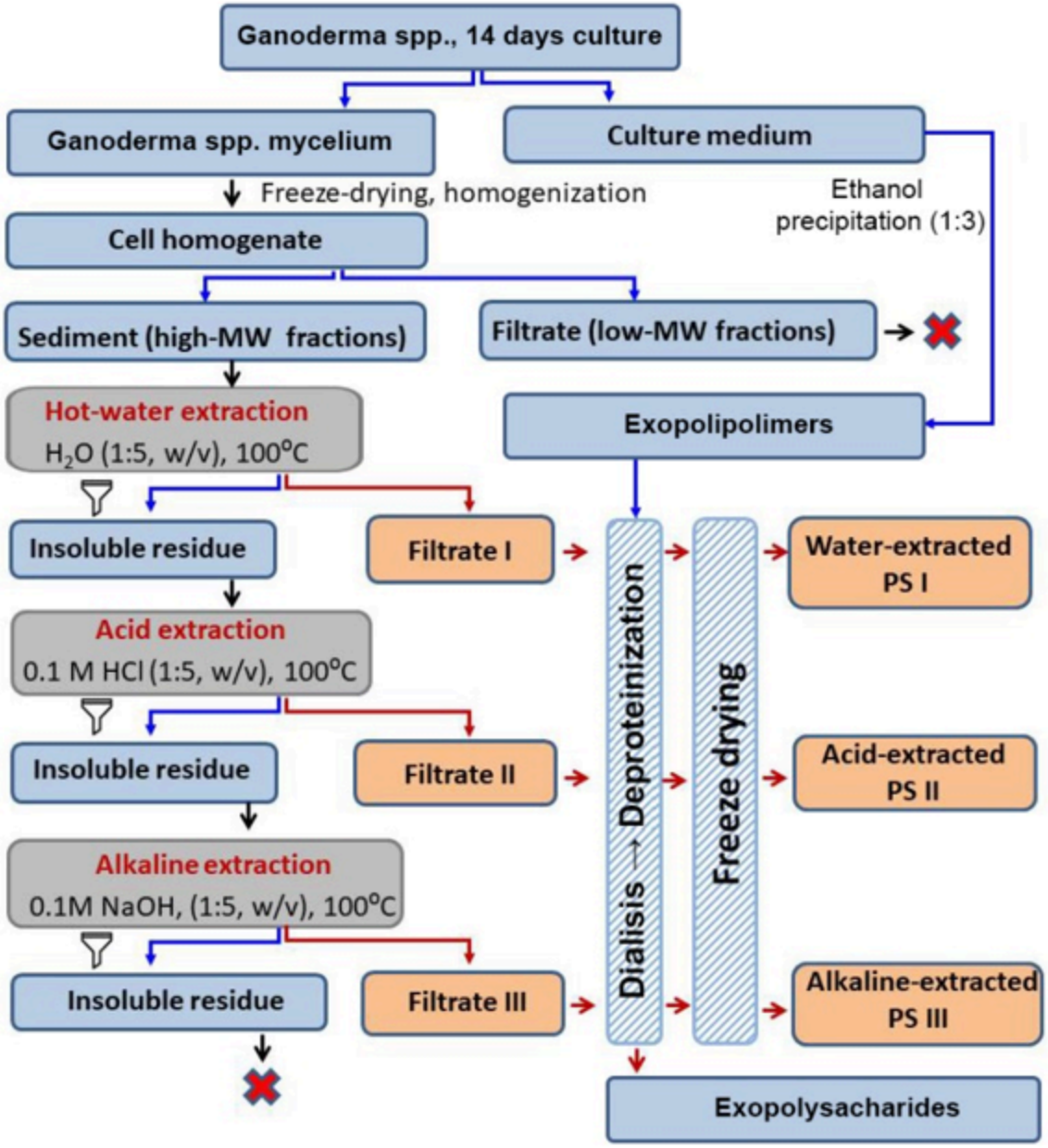


Figure 3

Workflow for the isolation and purification of intracellular (IPS I - IPS III) and extracellular (EPS) polysaccharides from *Ganoderma* spp. cultivated under submerged conditions. The scheme includes sequential hot-water, acid, and alkaline extractions of the mycelial biomass followed by purification steps (deproteinization, dialysis, and freeze-drying). Red crosses (×) indicate discarded fractions.

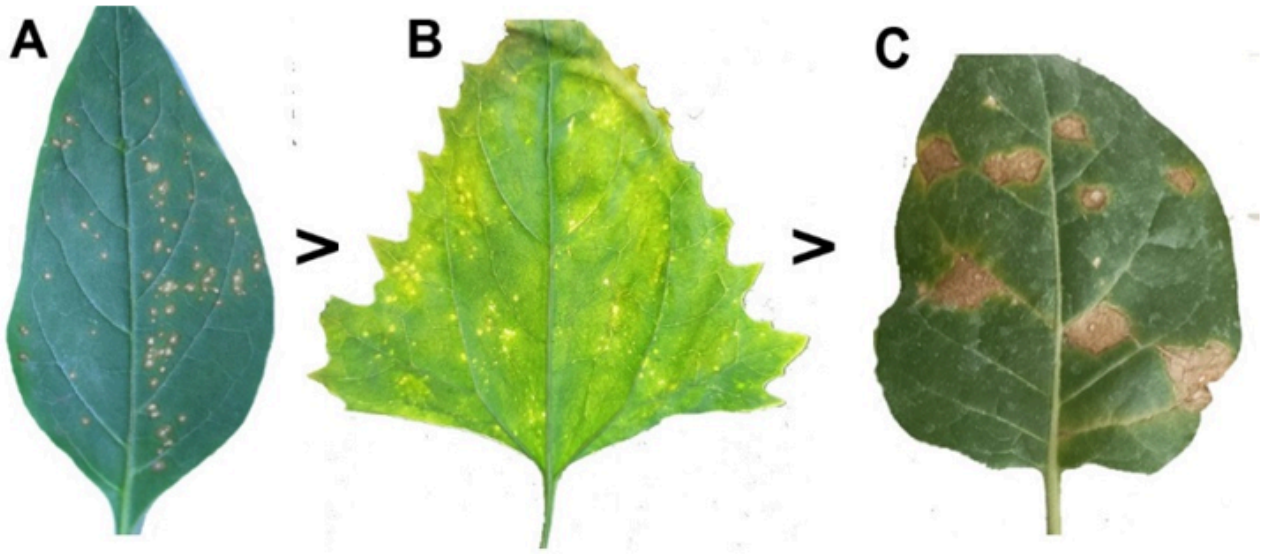


Figure 4

Local symptoms induced by TMV in indicator plants with different capacities for localization of viral infection: (A) *Datura stramonium*; (B) *Chenopodium amaranticolor*; (C) *Nicotiana sylvestris*

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