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Isolation and Evaluation of *Ganoderma lucidum* from Uttarakhand, India

Shilpi Rawat*

Department of Plant Pathology, College of Agriculture, GBPUAT, Pantnagar, India

**Corresponding author*

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A B S T R A C T

Ganoderma lucidum a species of class Basidiomycetes, belongs to the family polyporaceae (Ganodermataceae) of the order Aphyllophorales is one of the most popular medicinal mushrooms. Different isolates were collected, cultured and identified on the basis of basidiocarp and basidiospore morphology. The cultures of different isolates were studied for their radial growth at different media, temperature and pH. The study depicted that Malt extract agar media, temperature range of 25-35°C and acidic pH 5.0-6.0 were conducive for the mycelial growth of all *Ganoderma lucidum* isolates.

Introduction

Ganoderma lucidum (W.Curt: Fr.) P.Krast is one of the most popular mushrooms in oriental medicine. It is known as "Ling Zhi" in China and "Reishi" or Mannentake in Japan means "Herb of Spiritual potency" (Wagner *et al.*, 2003). In India, it is also called "jarhphorh" while in Haryana, popularly called "Satpatra" and "Hirdo". In India, ethno-medicinal value of *Ganoderma lucidum* was first reported by Harsh and coworker in 1993. Current world production of *G. lucidum* is around 6000 tones, half of which comes from China (Verma and Prasad, 2010). World trade in this mushroom is in the range of 1.5 billion US$, while it is about Rs.120 crore per annum in India (Geetha *et al.*, 2012). It is one of the most desired medicinal mushroom and has been used for more than 200 years. It is a popular remedy to treat condition like chronic hepatitis, hypertension, cancer, blood pressure, rheumatism, heart problem, paralysis, ulcer, arthristis, tiredness, hepatitis A, B, C, sterility, psoriasis, mumps, epilepsy and alcoholism. Products are available in various forms such as powders, tablet, capsule and syrups. This is probably the first medicinal mushroom to gain importance in India. *Ganoderma lucidum* has been cultivated by using several different substrates and by maintaining growth parameters such as temperature, relative humidity, water content, air, pH and light intensity (Changs and Miles, 2004). For this reason, it is very important to evaluate these factors for the optimal mycelial growth of

Ganoderma lucidum. In this study the mycelial growth of *Ganoderma lucidum* was observed on different medias as Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Czapek-Dox Agar (CDA), Sabouraud's Dextrose Yeast Agar (SDYA) and Wheat Extract Agar (WEA) at room temperature (25°C).

The mycelial growth was observed at temperature ranges viz. 15°C, 20°C, 25°C, 30°C, 35°C and pH levels viz. 4, 5, 6, 7,8 on MEA media. Present work was undertaken with the objective of evaluation of different factors for the optimal mycelial growth of *Ganoderma lucidum* and to identify the best isolates.

Materials and Methods

Collection and isolation of *Ganoderma lucidum*

Fruiting bodies of *Ganoderma lucidum* were collected from the forest areas of Almora (GA), Mukteswar (GM), Dehradun (GD), Pantnagar (GP) and Kashipur (GK) of Uttarakhand during rainy season and isolation of the collected specimens was made using tissue culture technique. The basidiocarps were washed twice with sterilized distilled water and dried with the help of sterilized Whatman filter paper under asepatic condition in the laminar flow inoculation chamber. Bits (3-4 numbers) from internal tissues of basidiocarp were taken with the help of sterilized forceps and were placed on the sterilized Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) media in the petri plates. Inoculated petri plates with the bits of fungal tissues were incubated at 25 ± 1ºC for a week for their growth. The culture tubes were inoculated from full grown fungual culture and incubated at above temperature. The full grown fungal culture tubes were kept in refrigerator for further studies.

Morphological characterization of *Ganoderma lucidum* isolates

Collected fruiting bodies of *Ganoderma lucidum* were studied for their morphological characteristics. Macroscopic features such as presence/absence of stripe, color, size and shape of fruiting bodies were recorded. Measurement of basidiospores were carried under compound light microscope using an eyepiece graticule calibrated with a stage micrometer.

Diametric growth of different isolates on different media, temperatures and pH

The experiment was carried out using five different media viz. Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Czapek-Dox Agar (CDA), Sabouraud's Dextrose Yeast Agar (SDYA) and Wheat Extract Agar (WEA) at room temperature (25°C). Petri-plates containing 25 ml of the medium were inoculated at the centre with 7 mm diameter disc of actively growing mycelium of *G. lucidum* isolates under aseptic conditions.

The isolates were inoculated in the petri plate containing 25ml of MEA media at varying temperature ranges viz. 15°, 20°, 25°, 30° and 35°C. The method was same as followed for temperature evaluation but at varying pH levels viz. 4, 5, 6, 7 and 8 on the MEA medium. pH was adjusted using concentrated HCl and concentrated NaOH solutions for lower and higher pH values, respectively. In all the experiments each treatments was replicated three times for each isolate and observations were recorded at two days interval upto full growth is observed in any one of the petri plates.

Statistical analysis

The data was analysed using three factorial CRD and the difference among mean value

was tested by using critical differences (CD) values at 5% level of probability.

Results and Discussion

Morphological characterization of *Ganoderma lucidum* isolates.

Isolate GA: Fruiting bodies were large, irregular in shape, copper red in colour, lateral surface appeared glossy, varnished, hard and thick. Basidiospores were ellipsoid, double walled, 6.0 - 8.9µm x 10 - 12µm in size.

Isolate GM: Fruit bodies were kidney-shaped along with long thick corky, hard stipe of dark brown in color. Fruit bodies were blackish-red in color. Basidiospores were ellipsoid, double walled and size varied between 6.84-7.37µm x 10.26 - 11.05µm.

Isolate GD: Fruit bodies were irregular with a thin margin and golden brown in colour with shiny appearance. Basidiospores were ellipsoid, cut flat at top, thick walled and 6.24-9.08µm and 9.11 - 10.00µm in size.

Isolate GP: Fruit bodies were kidney shaped, thick and broader margin. Color of fruit bodies were golden brown at centre, with a cream light yellow margin. Basidiospores ovate, with a rounded base and truncate to narrowly rounded apex, double walled and size in the range of 6.5 - 8.0µm x 10.0 -12.0µm

Isolate GK: Large fruit bodies with irregular shape and thick, broader margin. Dark black brown fruit bodies with wavy light yellow to cream margin, appearance like a floral pattern, found attached to the base of the tree with a dark thick pileus. Basidiospores were globoid, double walled and size of 7.5µm x 11.24µm.

The basidiocarp and basidiospore morphology has also been studied by Pegler and Young (1973) and Adaskaveg and Gilbertson (1986).

Diametric growth of different isolates on different media, temperatures and pH

Among the media tested MEA exhibit maximum average mycelial growth (7.64cm) followed by PDA (5.64cm), SDYA (3.46cm), WEA (2.92cm) and least in CDA (2.34cm) on 8th day of observation by all the isolate and differ significantly with each other. On MEA isolate GA showed maximum (full growth) diametric growth of mycelium (9.0cm) and different significantly from other isolates. Next to this, isolates GP gave mycelial growth of 7.73cm on 8th day and found to be at par with isolate GD (7.66cm) and differ significantly with other isolates. Lowest mycelial growth (6.50cm) was exhibited by GK followed by GM (7.30cm) on 8th day observation. However, on PDA average highest mycelial growth (6.90cm) was recorded in isolate GA which was at par with GP showing mycelial growth of 6.83cm and found significantly superior than other isolates. The next isolate was GD (5.16cm) followed by GK (4.90cm) and minimum mycelial growth was recorded in isolate GM (4.40cm) which differs significantly from others (Table 1–3).

On CDA medium isolate GA here also exhibited maximum mycelial growth of 2.83cm on 8th day and found to be at par with GP (2.76cm). Other isolates also exhibited significant variation to each other giving diametric growth range from 1.86cm to 2.23cm. On SDYA, isolate GA gave mycelial growth of 4.66cm which was found significantly superior to all other isolates. Isolate GP gave mycelial growth of 3.90cm which differs significantly with isolates GK (3.13cm), GD (2.90cm) and GM (2.70cm) which exhibited slow growth rate. On WEA isolate GA here also exhibited maximum mycelial growth of 3.46cm on 8th day which was at par with isolate GP (3.33cm) and differ significantly from other isolates.

Table.1 Diametric growth of *Ganoderma lucidum* (in cm) isolates on different media

	2nd days					Mean	4th days					Mean	6th days					Mean	8th days					Mean
Media	GA	GM	GK	GP	GD		GA	GM	GK	GP	GD		GA	GM	GK	GP	GD		GA	GM	GK	GP	GD	
MEA	3.50	1.00	0.08	2.16	1.36	1.76	5.70	2.50	3.00	3.08	2.66	3.53	7.20	4.04	4.8	6.23	4.80	5.48	9.00	7.30	6.50	7.73	7.66	7.64
PDA	2.16	0.00	1.00	1.56	1.00	1.14	3.73	1.26	2.30	2.96	2.33	2.52	5.73	3.06	3.50	5.50	3.86	4.33	6.90	4.40	4.90	6.83	5.16	5.64
CDA	0.56	0.00	0.00	0.00	0.00	0.11	1.23	0.00	0.73	1.13	0.83	0.78	2.30	0.83	1.16	1.90	1.50	1.54	2.83	1.86	2.03	2.76	2.23	2.34
SDYA	2.03	0.00	0.00	0.86	0.00	0.58	2.70	0.00	0.90	1.90	0.86	1.27	3.70	1.40	2.03	2.83	1.73	2.34	4.66	2.70	3.13	3.90	2.90	3.46
WEA	1.60	0.00	0.73	0.86	0.53	0.76	1.96	0.66	1.66	1.66	1.06	1.40	2.90	1.8	2.4	2.8	1.8	2.36	3.4	2.40	3.03	3.33	2.40	2.92
Mean	1.97	0.20	0.50	1.09	0.58		3.06	0.88	1.72	2.28	1.55		4.36	2.30	2.79	3.85	2.74		5.37	3.73	3.92	4.91	4.07	
CD at 5% Media × Isolates 0.07																								
Media × days 0.68																								
Isolates× days 0.68																								
Media × Isolates × days 0.15																								

Table.2 Diametric growth of *Ganoderma lucidum* (in cm) isolates on different temperature

	2nd days					Mean	4th days					Mean	6th days					Mean	8th days					Mean
Temperate	GA	GM	GK	GP	GD		GA	GM	GK	GP	GD		GA	GM	GK	GP	GD		GA	GM	GK	GP	GD	
15°C	1.36	0.89	1.10	1.43	0.43	1.04	2.53	2.33	2.33	2.43	1.73	2.28	3.76	3.66	3.73	3.40	2.96	3.50	5.63	4.93	5.10	4.76	4.50	4.98
20°C	2.20	0.63	0.89	2.03	1.46	1.46	3.13	2.16	2.16	3.66	2.90	2.80	5.53	4.20	5.40	5.53	4.93	5.12	8.23	6.56	7.16	7.70	7.06	7.34
25°C	2.53	1.00	1.30	1.40	1.40	1.52	3.73	3.46	4.26	3.73	3.40	3.72	6.26	5.26	6.63	6.80	6.33	6.26	9.00	8.23	9.00	8.83	8.53	8.72
30°C	0.30	0.00	0.00	0.96	0.23	0.30	2.13	0.70	0.86	2.46	1.63	1.56	3.53	2.36	2.56	3.76	2.86	3.02	4.80	3.80	4.36	5.10	4.16	4.44
35°C	0.36	0.00	0.00	0.70	0.33	0.28	1.80	0.33	0.43	1.56	1.73	1.17	2.96	1.83	1.80	2.96	2.46	2.40	4.23	3.00	3.26	3.93	3.46	3.58
Mean	1.35	0.56	0.66	1.30	0.77		2.66	1.80	2.01	2.77	2.28		4.41	3.46	4.02	4.49	3.91		6.38	5.30	5.78	6.06	5.54	
CD at 5% Temperature × Isolates 0.09																								
Temperature × days 0.08																								
Isolates× days 0.08																								
Temperature × Isolates × days 0.19																								

Table.3 Diametric growth of *G. lucidum* (in cm) isolates on different pH at temperature 25°C

	2nd days					Mean	4th days					Mean	6th days					Mean	8th days					Mean
pH	GA	GM	GK	GP	GD		GA	GM	GK	GP	GD		GA	GM	GK	GP	GD		GA	GM	GK	GP	GD	
4.0	1.40	0.7	1.36	1.70	1.06	1.24	2.23	1.23	2.33	2.90	2.83	2.30	4.63	2.96	3.90	4.40	3.90	3.96	6.23	4.23	5.50	5.70	5.43	5.42
5.0	1.33	1.56	1.80	2.23	2.00	1.78	3.16	2.66	2.73	4.46	3.60	3.32	5.86	4.56	4.46	6.90	4.93	5.34	8.16	6.03	6.63	8.83	7.60	7.45
6.0	1.33	1.06	1.40	2.30	1.83	1.58	3.53	2.36	3.46	4.13	4.10	3.52	6.53	4.13	5.43	7.13	6.90	6.02	8.23	6.63	6.90	8.83	8.16	7.75
7.0	1.53	1.30	1.70	1.90	1.86	1.66	2.60	2.53	3.13	3.86	3.60	3.14	5.03	4.03	3.83	5.20	5.40	4.70	5.86	5.46	5.23	6.23	6.13	5.78
8.0	1.06	0.36	1.30	1.36	1.23	1.06	1.96	1.46	1.76	2.06	2.30	1.91	3.13	2.53	2.70	3.26	3.43	3.01	4.63	3.70	3.80	4.36	4.46	4.19
Mean	1.33	0.99	1.51	1.90	1.60		2.70	2.05	2.68	3.48	3.24		5.04	3.64	4.06	5.38	4.91		6.62	5.21	5.61	6.79	6.36	
CD at 5% pH × Isolates 0.33																								
pH × days 0.30																								
Isolates× days 0.30																								
pH × Isolates × days 0.67																								

Table.4 Means for main effects

Media	Mean	Temperature	Mean	pH	Mean
MEA	4.60	15°C	2.95	4.0	3.23
PDA	3.41	20°C	4.18	5.0	4.47
CDA	1.19	25°C	5.05	6.0	4.72
SDYA	1.91	30°C	2.33	7.0	3.82
WEA	1.85	35°C	1.86	8.0	2.54
CD at 5%	**0.03**	**CD at 5%**	**0.04**	**CD at 5%**	**0.05**
GA	3.69	GA	3.70	GA	4.03
GM	1.78	GM	2.76	GM	2.97
GK	2.23	GK	3.12	GK	3.47
GP	3.03	GP	3.66	GP	4.38
GD	2.23	GD	3.12	GD	3.92
CD at 5%	**0.03**	**CD at 5%**	**0.04**	**CD at 5%**	**0.05**
D2	0.87	D2	0.91	D2	1.46
D4	1.90	D4	2.30	D4	2.84
D6	3.21	D6	4.06	D6	4.60
D8	4.40	D8	5.81	D8	6.12
CD at 5%	**0.03**	**CD at 5%**	**0.38**	**CD at 5%**	**0.04**

Isolates GK showed mycelial growth of 3.03cm and exhibited significant variation to other isolates. Isolates GD and GM each showed minimum growth of 2.34cm only.

All the five isolates of *G. lucidum* grew well on Malt Extract Agar followed by Potato Dextrose Agar and isolate GA with mycelial growth of 5.37cm was found to be superior followed by isolate GP (4.91cm) on 8th day observation.

MEA with mycelial growth of 4.60cm on 8th day (4.40cm) was found to be best for isolate GA (3.69cm). Malt Extract Agar (MEA) has been reported as the most suitable medium for the growth of *G. lucidum* by Adaskaveg and Gilbertson (1986), Shukla and Uniyal (1989), Khara *et al.,* (1997) and Mishra (2010).

Among the temperatures, all the isolates showed average maximum mycelial growth at 25°C (8.72cm) followed by 20°C (7.34cm) and minimum at 35°C (3.58cm) on 8th day observation. At 25°C temperature on 8th day, isolates GA and GK exhibited maximum mycelial growth (9.0cm each). Isolates GP (8.83cm) and GD (8.53cm) were at par to each other but differs significantly with isolated GM (8.23cm) which exhibited lowest mycelial growth on 8th day. However, at temperature 20°C highest average mycelial growth (8.23cm) was recorded from isolate GA which was found significantly superior than other isolates. The next isolate in order to superiority was GP giving 7.70cm mycelial growth followed by isolate GD (7.16cm) and GK (7.06cm) which were at par to each other. Here also, isolates GM produces minimum growth (6.56cm) only. At 15°C temperature, isolates GA gave average mycelial growth of 5.63cm and found superior to all other isolate. Isolates GK gave mycelial growth of 5.10cm which differs significantly with all others. Other isolates GP (4.76cm), GD (4.50cm) and GM (4.93cm) exhibited minimum growth on 8th day observations. At 30°C, isolate GP exhibited highest mycelial growth (5.10cm) on 8th day observation and differ significantly from other isolates followed by isolate GA showed 4.80cm mycelial growth. Other isolates also exhibited significantly variation to each other giving diametric mycelial growth ranges from 3.80cm - 4.36cm. Minimum growth of all the isolates were recorded at 35°C temperature on which highest mycelial diametric growth (4.23cm) obtained from isolate GA with followed by isolate GP (3.93cm). Minimum growth 3.0cm and 3.26cm were recorded from isolated GM and GD, respectively. All the isolates varies significantly to each other. Increasing the temperature 35°C or above it, resulted into decrease in average growth rate.

Temperature range of 20°C-25°C was found to best for isolate GA with mycelial growth of 6.38cm followed by isolate GP (6.06cm) on 8th day observation. Optimal mycelial growth (5.05cm) was exhibited at temperature of 25°C on 8th day (5.81cm) where isolate GA gave maximum mycelial growth of 3.70cm (Table 4). The optimum temperature of 20°C-25°C has been reported to give maximum mycelial growth by Song *et al.,* (2007) and Nasreen *et al.,* (2005).

For the pH study, data showed that all the isolates exihibited maximum mycelial growth (7.75cm) at pH 6.0 followed by pH 5.0 (7.45cm) and least mycelial growth (4.19cm) at pH 8.0 by all the isolates on 8th day of observation and differ significantly with each other. At pH 6.0, isolates GP gave maximum mycelial growth (8.83cm) and differs significantly from all other isolates. Mycelial growth by isolate GA (8.23cm) and GD (8.16cm) were at par to each other but differs significantly with all other isolates. Minimum growth of 6.63cm was exhibited by isolate GM. At pH 5.0 also, isolate GP gave maximum diametric mycelial growth

(8.83cm) and differs significantly with all other isolates. Isolate GA was found next to isolate GP with 8.16cm of diametric mycelial growth and differs significantly with all other isolates. Minimum growth of mycelium 6.03cm per was recorded in isolate GM. At pH 7.0, the isolate GP showed diametric mycelial growth of 6.23cm on 8th day observation, which was at par with isolate GD (6.13cm) but differs significantly from other isolates. Isolates GA and GM resulted in diametric mycelial growth of 5.86cm and 5.46cm and minimum diametric mycelial growth of 5.23 cm was exhibited by isolate GK on 8th day. At pH 8.0 on 8th day, isolates GA and GD were at par to each other with 4.63cm and 4.64cm of diametric mycelial growth but differs significantly to other isolates.

Isolates GP, GK and GM showed diametric mycelial growth of 4.36cm, 3.80cm and 3.70cm, respectively. At pH 4.0 on 8th day, isolate GA exhibited diametric mycelial growth of 6.23cm which differs significantly from all other isolates. Isolates GP, GD, and GK exhibited diametric mycelial growth in the range of 5.43cm to 5.70cm and were at par to each other. Minimum average mycelial growth of 4.23cm was exhibited by isolate GM.

All the isolates preferred acidic pH 5.0-6.0 for their growth. Isolate GP was found to be superior with maximum mycelia growth (6.79cm) followed by isolate GA (6.62cm). pH of 6.0 (4.72cm mycelial growth) was superior for all the isolates and isolate GP (4.72cm) on 8th day (6.12cm) gave maximum mycelial growth. It has also been reported that *Ganoderma lucidum* preferred acidic pH by Venkatarayan (1935), Triratana *et al.*, (1991), Khara *et al.,* (1997) and Rai (2003). pH 5 is found most suitable for the mycelial growth of *Ganoderma lucidum* by Mishra (2010) and Nasreen *et al.,* (2005).

Based on the present study it can be concluded that different ganoderma isolates favours malt extract agar media with a temperature of 20°C-25°C and pH of 5.0- 6.0 for the optimal mycelial growth and among the five different isolates, isolate GA and isolate GP was found to be superior on 8th day observations.

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References

Adaskaveg JE, Gilbertson RL 1986. Cultural studies and genetics of sexuality of *Ganoderma lucidum* and *G. tsugae* in relation to the taxonomy of the *G. lucidum* complex. Mycologia 78: 694-704.

Chang ST, Miles PG 2004. Mushrooms: Cultivation, nutritional value, medicinal effect and environmental impact. (2nd edition) Boca Raton, CRC Press.

Geetha D, Suharban M, Gokulapalan, Rani CVD 2012. A low cost technology for cultivation of *Ganoderma lucidum.* Mushroom Research 21(1): 49-53.

Harsh NSK, Rai BK, Tiwari DP 1993. Use of *Ganoderma lucidum* in folk medicine. Indian Journal of Tropical Biodiversity 1(4):324-326.

Khara HS, Singh Jatinder 1997. Diagnosis of *G. lucidum* root rot of trees, the cultural characteristics, spore germination and percentage of root-decay. Plant Disease Research 12(2): 108-112.

Mishra KK, Singh RP 2010. Cultural and biochemical variability amongst indigenous *Ganoderma lucidum* isolates from Uttarakhand. Mushroom Research 19: 74-81.

Nasreen Z, Kausar T, Nadeem M, Bajwa R 2005. Study of different growth parameters in *Ganoderma lucidum*. Mycologia Aplicada International 17 (1): 5-8.

Pegler DN, TWK Young 1973. Basidiospiores form in the british species of *Ganoderma* Karst. Kew Bull 28:351-369

Rai RD 2003. Successful cultivation of the medicinal mushroom Reishi (*Ganoderma lucidum)* in India. Mushroom Research 12(2):87-91.

Shukla AN, Uniyal K 1989. Antagonistic interactions of *Ganoderma lucidum* (Leyss.) against some soil micro-organisms. Current Science 58(5):265-267.

Song M, Kim N, Lees, Hwang S 2007. Use of Whey permeate for cultivating ganoderma lucidum mycelia. Journal of Dairy Science 90:2141-2146.

Triratana S, Jhaithatgoon S, Gawgla M 1991.Cultivation of *G. lucidum* in saw-dust bags. Science and cultivation of edible fungi, Maher (ed) Balkema, Rotterdam 567-572.

Venkatarayan SV 1935. The biology of *G. lucidum* on areca and coconut palms. Phytopathology 26: 153-175.

Verma A, Prasad R 2010. Induced single spore germination in *Ganoderma lucidum*. Mushroom Research 19 (2):68-73.

Wagner R, Mitchell DA, Sassaki GL, Amazonas MAL deA, Berovic M 2003. Current technique for the cultivation of *Ganoderma lucidum* for the production of biomass, ganoderic acid and polysaccharides. Food Technology and Biotechnology 41(4): 371-382.

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