ISSN No: - 2456 - 2165

In-Vitro Propagation of Ganoderma Lucidum – A Medicinal Mushroom in Different Culture Medium

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Abstract :- The present investigation has been done to develop an improved low cost in-vitro culture protocol for large-scale mycelium growth possessing the ability to bear fruiting bodies, important for artificial cultivation of *Ganoderma lucidum*. *Ganoderma lucidum* (Fr.) P. Karst is one of the most popular mushrooms in traditional Chinese medicine (TMC) which has a long fascinating history dating back over two thousand years in China. Besides infrastructure and raw material, healthy vegetative mycelial culture is required to produce quality spawn. In the proposed study, in-vitro propagation of the pure culture of G.lucidum is done on potato dextrose (PDA), Oatmeal agar, and Wheat extract agar slant in culture tubes. For better commercial viability, four groups of Ganoderma Lucidum culture for each of the media were prepared. These groups were inoculated through a sample of mother culture obtained from NRCM, Solan. Group 1 was inoculated by mycelium of mother culture tube, group-2 was inoculated with mycelium as well as spores of fruiting body, group -3 was inoculated by spores of mother culture tubes as well as spores from another sample. It means we started with two sample of culture tubes. The produced cCulture tubes were subjected to incubation for 10 to 14 days in aseptic conditions at 320 -350 C to achieve a good mycelial growth. It was found that the group-4 culture could produce a fruiting body after transplantation on sterilized substrate prepared for artificial GL cultivation. Further, PDA was observed to be the most suitable culture media for the propagation of Ganoderma lucidum. After experiment on the different groups and evaluations based on the standard metrics, spore of different strains has been found most suitable for commercial viable production of GL fruiting body.

Keywords: *Ganoderma lucidum, (PDA) Potato dextrose Agar, Dikaryon, heterothallic, Oatmeal agar, Wheat extract agar.*

I. INTRODUCTION

Ganoderma lucidum is a lignin-degrading white rot fungus on hardwoods. It has a long fascinating history in China dating back over two thousand years. This large fungus is acclaimed as mushroom of immortality and is portrayed as number one medicinal mushroom in China. In Japan, it is known as Reishi, while in China as Ling Zhi. The most popular authority in the west on Eastern medicine Dr. Andrew Weil advised to consume Reishi in daily routine to prevent cancer. It is safe for long-term use and known as an immune system enhancer and immune modulator with health benefits. In Asia, it was used in the treatment of several diseases for thousand of years as reported in Shen Nong's Materia Medica.

However, an increasing systematic research into the Ganoderma lucidum active compounds elucidates numerous pharmacological effects such as antitumor, immunomodulatory, cardiovascular, hepatoprotective effect. Modern uses of Ganoderma lucidum therefore include treatment of coronary heart diseases, arteriosclerosis, arthritis, hepatitis, bronchitis, asthma, hypertension, obesity, cancer and gastric ulcer. New reports emphasize its potential in treatment of viral infection. It is more recognized for its balancing properties. Latest available estimates put the annual value of *G. lucidum* products worldwide at more than US $1.6 billion. This increased demand has stimulated improvements in artificial cultivation methods. The quality and content of physiologically active substance vary from strain to strain and also depends upon location, cultural condition & the processing procedures as mycelia growth do nit need sunlight but oxygen is required since G.lucidum is strict aerobic. Diverse group of chemical compounds with pharmacological active constituents of Ganoderma lucidum are triterpenoids, polysaccharides, proteins, amino acids, alkaloids, steroids, lactones, fatty acids and enzymes. The most important pharmacologically active constituents are triterpenoids and polysaccharide. As G. lucidum is very scarce in nature, artificial cultivation has become essential to meet the demands of international markets. For artificial cultivation superior quality having vigorous and fast growing *Ganoderma lucidum* culture is required in large scale. Even the mycelium contains several bioactive substances. Hence, developing of a scientifically improved low cost in-vitro culture protocol for large-scale mycelium growth required for the good of the society. Present paper is all about the in- vitro cultural propagation of G. lucidum and commercial production of thread like mycelium. Multispores cultures were also used for conservation of wild germplasm and serve as starter in mushroom cultivation.

II. MATERIALS AND METHODS

A. Culture

The most accepted indigenous strain of Ganoderma lucidum was obtained from National Research Center for mushroom

ISSN No: - 2456 - 2165

Solan, Himachal Pradesh. The test tube having culture of Ganoderma lucidum was true to type genuine DNA finger printed available in the NRCM's mushroom Bank.

B. Culture medium

Peeled & diced Potato :200g
Dextrose : 20g
Agar-agar powder : 20g
P^H : 7

C. PDA(Potato dextrose Agar)

Potato dextrose Agar medium was used in the present study. 200grams of Potatoes were washed cut into small pieces (3-4 cm) peeled and boiled in distilled water for 20 minutes. The liquid broth was strained through Cheesecloth and the potatoes cubes were discarded. The volume of this decanted broth was raised to one liter. 20 grams Dextrose and 20 grams Sabouraud agar powder was then added. Homogenized the medium by continuously stirring and sterilized by autoclaving at 15 pounds per square inch (100 kPa) for 15 minutes. Green potatoes should not used as they contain anti-fungal alkaloids, which may be harmful.to mushroom mycelium. PH of the medium was adjusted to 6.2 before sterilization. It has been observed that the pH declines approximately 0.5 upon autoclaving. After autoclaving the pH becomes 5.6, which is most suitable for mushroom mycelium. The medium was first sterilized in 500 ml conical flask 15 to 20 ml of lukewarm sterilized culture media was poured in pre- sterilized petriplates. The culture tubes and conical flask was plugged with non-absorbent cotton and autoclaved under moist steam at 15 pound per square inch pressure for 25 to 30 minutes. After sterilization, culture tubes were placed in slanting position to provide more surface area for vegetative mycelia growth. Culture media is allowed to solidify in culture tube. Similarly, two more culture media were prepared.

D. Oat Meal Agar

Oat-meal flakes : 30g
Agar- agar powder: 20g
Water: 1 liter
p^H : 7

Oatmeal flakes were boiled in sufficient water for 2 h. The volume of the supernatant was raised to one lite. Stirring mixed agar-agar powder and pH was adjusted

F. Wheat Extract Agar
Wheat grains : 32g
Agar-agar powder: 20g
Water: 1 liter

P^H : 7

Wheat grains are boiled in water for 1-1/2 h. They were filtered through muslin cloth and grains were discarded. The volume of grain extract was raised to 1 liter with water and then agar-agar powder was added by stirring continuously and pH was adjusted.

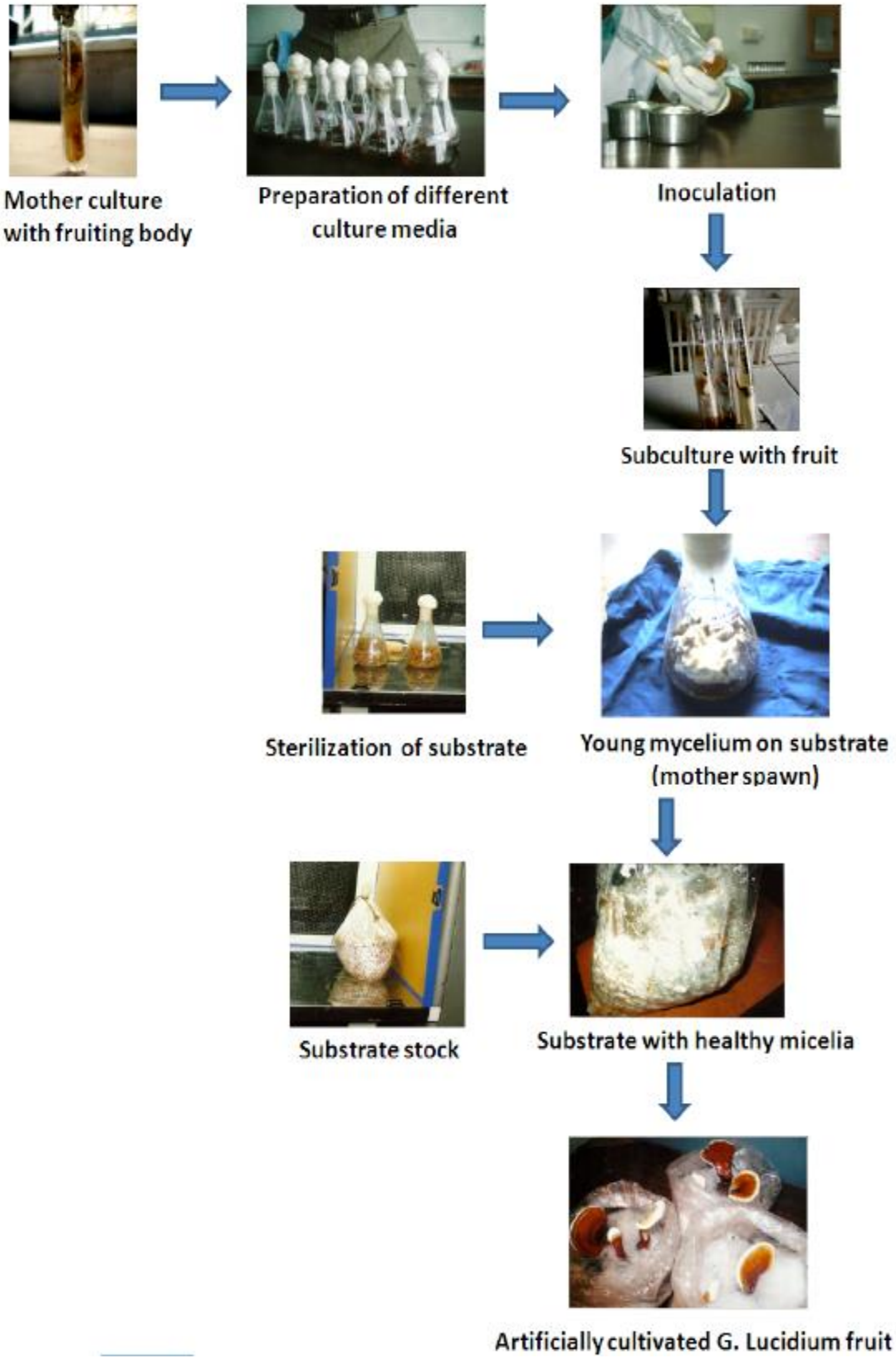
G. Inoculation
Inoculation was done inside an inoculation room in the chamber of laminar flow. Inoculation room was sterilized with UV light and 95% ethyl alcohol. 40 culture tubes were inoculated in first attempt. Group -1 Ten culture tubes each having culture media (mentioned above) were inoculated by young mycelium from growing edges having size of 8 mm. Group -2 another Ten sets of culture tubes were inoculated using the spores from the fruiting body of mother culture tubes . Group -3 third ten sets of culture tubes were inoculated by both with small piece of mycelium as well as spores from the fruiting body of mother culture. Group 4 having ten culture tubes were inoculated by spores of the fruiting body mother culture tube. This time more than two mother culture tubes were used. They were observed every day for their morphological responses.

F. Spore procurement
For multiple spores, culture spores were procured from the mother culture tube by making aqueous suspension and transferring aquatic suspension to new culture tubes under aseptic conditions.

G. Procedure
The procedure adopted is shown in Scheme 1. In vitro propagation of the pure culture of G.lucidum was done on potato dextrose (PDA), Oatmeal agar, & wheat extract agar slant in culture tubes. Four sets each having 10 culture tubes containing different culture media was used. Set-1 was inoculated by 6mm piece of fungal mycelium. Mycelium as well as spores was used to inoculate culture tubes of set -2. Spore of the fruiting body of mother culture tube was used to inoculate set- 3. Spores of more than two culture tubes were used to inoculate culture tubes of set -4. Inoculated culture tubes were incubated initially at 300 to 32 0 in a BOD incubator. Within 4 to 5 days new mycelium growing over media was observed. These culture tubes were incubated further to 10 to 14 days in aseptic condition at 320 -350 C to achieve a good mycelia growth. Inoculated culture tubes were incubated initially at 30-32oC in a BOD incubator.

Scheme

ISSN No: - 2456 - 2165



Scheme 1: Procedure for artificial culture of G. Lucidium

ISSN No: - 2456 - 2165

III. RESULTS AND DISSCUSSIONS

Within 4 to 5 days new mycelium growing over media was observed. These culture tubes were incubated further to 10 to 14 days in aseptic condition at 320 -350 C to achieve a good mycelia growth. It was found that culture tubes of group-4 were able to produce fruiting body after transplanting it on sterilized substrate prepared for artificial cultivation(Fig-1, & 3).PDA was observed as a most suitable culture media for the propagation of Ganoderma lucidum (Table -1).

Table-1:- Relative growth of young mycelium inside culture tubes after inoculation.("- " No growth, "+ "weak growth, "++" healty growth, "+++" very healthy and vigourous growth.

	PDA	OMA	WEA
Set-1	-	-	-
Set-2	+	-	-
Set-3	+	-	-
Set-4	++++	+	++

Inoculation through spores of different culture was required to get the fruiting body as this particular mushroom is heterothallic hence they need to mate with opposite group to form dikaryon (Verma R.N et al. 2003). Rest of the culture tubes had mycelium run but no fruiting body. So, spore culture is recommended to get fruiting body for artificial cultivation to get enhanced yield and stronger variety. No mycelial growth was found in the tubes of set- 1. Weak and slow growth of young mycelium were observed in the culture tubes of set- 2 & set-3 where the different culture medium were inoculated by tissue (mycelium tip) and tissue (Mycelium tip)+spores(set-3). No growth on the tissue indicates that tissue is dead, probably due to heat of scalpel or keeping tissue very near to flame for long duration of transfer. Tissue culture may not grow on a medium due to contamination. S.K. Singh and M.C. Yadav of NRCM observed similar finding. Over 80% of the population in developing countries is estimated to depend upon traditional medicine for their daily needs. Alternative medicine is getting increasingly popular in developed countries, too. Therefore, it is advisable to develop protocol for rapid in-vitro regeneration of this medicinal mushroom. This will make traditional medicine affordable and accessible to society and can be used in combination with modern medicine. Further expansion of the market for *G. lucidum* products will require the introduction of more reproducible protocols for mushroom production and downstream processing to improve quality control and ensure enduring public trust.

IV. CONCLUSION

An improved low cost in-vitro culture protocol for large-scale mycelium growth required for artificial cultivation of Ganoderma lucidum has been developed. Most suitable media for in vitro propagation of Ganoderma lucidum was found to be PDA. The optimum temperature and pH was found to be around 30-320C and pH 5.6. This method can be applied for commercial production of G. Lucidium for use in pharmaceutical industry.

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