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Production of mycelial biomass and antibiotic by the mycelium of *Ganoderma lucidum* (Curt, : Fr.) by submerged fermentation

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In this experiment the production of mycelial biomass and antibiotic by *Ganoderma lucidum* (Curt, : Fr.) is reported. The fermentation was made in a fermentor for 30 days. The experimental data revealed that the mycelial growth was maximum at 30th day. The growth started very slowly from 2nd day along the incubation period. The pH of the medium changed to acidic side from 2nd day onwards, gradually went down to 5.6 on 16th day and reached 6.5 on 30th day. The mycelial growth was very good when the pH of medium was around 6.0 (i.e. 5.7 to 6.5). The antibiotic activity was found on and from 18th day onwards of fermentation and it was maximum at 30th day. The antibiotic activity was also not detected in the pH range up to 5.6. When the pH started to increase from 5.6 to 5.7 and onwards the antibiotic activity was detected. Polysaccharides and terpenoids were isolated having antibiotic properties.

Key words: *Ganoderma lucidum,* mycelial biomass, antibiotics

INTRODUCTION

In India, the knowledge of the application of mycelial extracts of higher fungi in the field of medicine dates back to vedic period, when the people pay attention to the use of crude extracts of mushrooms or higher fungi for the treatment of different ailments. Now a days researches are going on world wide for more utilization of this natural wealth for the benefit of mankind. Traditionally the edible or medicinal mushrooms are cultivated in solid cultures which takes several months to produce fruit bodies. and the bioactive metabolites produced from these fruit bodies can not retain consistent quality. In this context , the submerged culture offers potential advantages of faster production of both mycelial biomass with high nutrients and exopoly saccharides of medicinal importance in shorter period of time within a reduced space and gets lesser chances of contaminations (Kim *et al.,* 2002) with consistent quality (Kwon *et al.,* 2009); Anthony and Jehova (2009) have performed submerged liquid fermentation of *Ganoderma* spp. and *Pleurotus* spp. to obtain mycelial biomass and exopolysaccharides. According to Wagner *et al* (2003), the pharmacological effects of *Ganoderma* spp are attributable mainly to polysaccharides and triterpenoids showing antibiotic activity. Hassan *et al.* (2012) have obtained antibiotics from the submergd culture of *Flammulina velutipes.* Zhuang

et al (1993) heve observed antitumor activity of the polysaccharides from the biomass of *Pleurotus sajor-caju.* Tripura, the small hilly state of north-east India is rich not only in luxuriant vegetation, but also in varied mycoflora. The tribal people of this state are used to collect wild higher fungi for consumption and also apply in crude way for the treatment of different ailments. In the present investigation attempts have been made to collect the most commonly growing and used fungus *Ganoderma lucidum* (Curt, : Fr) from the forest bed of this state and to produce mycelial biomass by submerged fermentation in fermentor. The antibiotic property and isolation of terpenoids and polysaccharides are also done from the mycelial mass.

MATERIALS AND METHODS

During the rainy season, the fungus was found to grow on the infected logs on the forest beds of South Tripura. The fruit bodies were collected and immediately brought to the laboratory. Morphological and anatomical studies were done by taking hand sections and measurements of reproductive structures. Several tissue cultures were prepared from the freshly collected fruit bodies on PDA medium and stored in refrigerator at 10°C and maintained by regular subculturing at 30 days interval. The fungus was initially grown stationary in liquid synthetic medium i.e. glucose asparagine medium (Lilly and Barnett, 1951) in conical flasks for studying the growth of mycelia.

Preparation of inoculum for fermentation

A small portion of the actively growing mycelium in PDA was pounched out aseptically by a sterilized cork borer (5 mm dia) and was transferred aseptically to Erlenmeyer flask (150ml) containing 30ml of sterile-glucose asparagine medium. The inoculated flasks were in incubated for 7 days in complete darkness in shaking incubator (120 rpm) at 30°C. After harvesting, the mycelia were repeatedly washed with sterile distilled water to remove any trace of medium and were fragmented into small pieces aseptically in a waring blender. Then the fragmented mycelia were washed again with sterilized distilled water and suspended in phosphate buffer (pH 6.0) medium for 24 hrs to overcome the shock encountered during blending. From this fragmented mycelial suspension, 10 ml was used as inoculum.

Production of mycelial biomass

An optimal medium was prepared containing glucose - 30 g; peptone-1 g; yeast extract -2 g; KH_2PO_4, 0.5g; $MgSO_4$,$7H_2O$-0.1g; $FeCl_3$ - 0.01g; $ZnSO_4$-0.002g, $MnSO_4$- 0.002g; $CaCl_2$, - 0.05g and M_0O_3 - 0.001g per litre and distilled water was added to make it 1000 ml. The fermentor (IL) was dispensed with 300 ml of sterilized optimal medium which was adjusted to pH 6.0 with 0.2 M Phosphate buffer and inoculated with 10 ml mycelial suspension and incubated at 30°C with 150 rpm and aeration of 21 air/minute . 2 ml of Polyol antifoam was added to inhibit foaming during fermentation. The incubation was contained for 30 days and at every 2 days interval, three fermentors were harvested. During harvesting mycelia were separated from the medium by filtration through sintered IG3 disc using vacuum pump and harvested mycelia were washed three times with distilled water and then the entire biomass was air dried and ground in Wiley Mill attached with 60 mesh screen. The dry weight of the biomass was recorded. The antibiotic activity was determined by the standard plate diffusion assay method using *Bacillus subtilis* as test organism and measured by taking the diameter of inhibition zone.

Isolation of polysaccharide from mycelial biomass

The isolation of polysaccharide from the mycelial biomass of the test fungus was done following the methods of Mizuno *et al.* (1992) and Wang *et al* (1993). Mycelia of the test fungus were harvested aseptically from 30 day old liquid culture in fermentor (IL) by filtration through sintered IG3 disc using vacuum pump and the harvested mycelia were washed and the entire biomass was air dried and ground in Wiley Mill attached with 60 mesh screen. The powdered mycelial biomass (ca 500 g) was treated with 85 per cent ethanol (by 10 volumes) repeatedly (4 times) at room temperature. 30°C ± 2°C for 24 hrs to remove the lower molecular weight substances. The residue was then treated with hot water (100°C) for 3 hrs and filtered. The filtrate was collected nealtly and evaporated completely and it was Fraction 1 (F_1) of polysaccharide. The residue was treated with 1 per cent ammonium oxalate solution at 100°C for 6 hrs. The filtrate was collected and evaporated completely under reduced pressure. This was fraction 2 (F_2) of the polysaccharide. This residue

was further treated with 5 per cent sodium hydroxide solution at 80°C for 6 hrs. The filtrate was collected and evaporated completely under reduced pressure. This was collected as fraction 3 (F_3) of the polysaccharide. These crude fractions were used as polysaccharide in the experiments.

Isolation of terpenoids from mycelial biomass

The isolation of terpenoids from the sumerged mycelial biomass was done following the method of Shiao *et al* (1986), Anke and Werle (1980) and Chairul *et al* (1991). Mycelia of the test fungus were harvested aseptically from the 30 days old liquid culture in fermentor (IL) by filtration through sintered IG3 disc using vacuum pump and the harvested mycelia were washed at least three times with sterile distilled water in order to remove the adhering medium. Then the entire biomass was air dried and ground in Wiley mill and passed through 60 mesh screen. The mycelial powder (ca 500 g) was extracted with methanol. The methanolic extracted was concentrated under reduced pressure and the concentrated extract was dissolved in saturated sodium bicarbonate solution three times. The sodium bicarbonate solution was acidified with 10% HCl and extracted three times with ethyl acetate. The entire acidified ethyl acetate extract was chromatographed on a silica gel column with hexano-ethyl acelate (1:1) mixture and three fractions (F_1, F_2 and F_3) were collected. These crude fractions were used as terpenoids in all the experiments.

RESULTS AND DISCUSSION

Fresh fruit bodies of *Ganoderma lucidum* (Curt, : Fr:) were collected from the forest beds of South Tripura and the tissue culture were prepared and maintained on PDA slant. Inoculum of mycelial suspension was prepared in liquid glucose - aspergine medium. For submerged mycelial biomass, the fungus was grown in fermentor for 30 days. The antibiotic activity, during growth was measured by agar -plate diffusion method using *Bacillus subtilis* as test organism. Acidified and terpenoid were isolated and activity was tested using a no of bacterial strains. The data obtained were presented in Table 1 and 2.

Data in the Table 1 revealed that the mycelial growth of the test fungus was maximum at 30 day.The growth started very slowly from the 2nd day onwards and gradually increased linearly along with the incubation period upto 30 days. The pH of the medium changed to acidic from 2nd day onwards, gradually went down to 5.6 on16th day and again started to rise and reached 6.5 at 30th day. the growth was very good when the pH of the medium was around 6.0 (i.e. 5.7 to 6.5). The antibiotic activity was found on and from 18 day of fermentation and it was maximum at 30th day. The antibiotic activity was also not found in the pH range upto 5.6. When the pH started to increase from 5.6 to 5.7 and onwards the antibiotic activity was observed.

Table 1 : Data[a] (mean) showing the growth of mycelia and antibiotic activity of culture filtrate of *Ganoderma lucidum* in the optimal medium during fermentation

Days	pH of the medim[b]	Dry wt. of mycelum[c] (mg / 30ml)	Activity[d] (mm)
0	6.0	–	–
2	5.8	26±2	–
4	5.8	56±4	–
6	5.6	96±5	–
8	5.6	128±6	–
10	5.5	168±5	–
12	5.4	186±6	–
14	5.6	201±5	–
16	5.6	222±6	–
18	5.7	161±5	04±0.8
20	5.7	302±6	14±0.8
22	5.8	348±6	19±0.8
24	5.8	372±6	26±1.0
26	5.9	401±7	31±1.0
28	6.1	432±8	35±1.2
30	6.5	460±8	38±1.2

(–) indicate no activity.
[a] Data are mean of three replicates ± S.D.
[b] The pH of the medium was adjusted to 6.0 with 0.2 M phosphate buffer.
[c] Dry wt. of fittered washed mycelia grown in fermentor.
[d] The antibiotic activity was determined by standard plate diffusion assay method and presented as diameter of inhibition zone (mm)

The data in the Table 2 revealed that the terpenoids and polysaccharide, isolated from *Ganoderma lucidum* (Curt, : Fr.) were not at all active against *Mycobacterium phlei* and *Sarcina lutea*. Terpenoids were more inhibitory than the polysaccharide. The maximum inhibition was found in *Bacillus brevis* with terpenoid and minimum in *Corynebacterium insidiosum* . With polysaccharide the maximum inhibition was found in *Bacillus subtilis* and minimum in *Staphyllococus aureus.*

The present investigation regarding the production of mycelial biomass in submerged fermentation along with antibiotic production by *Ganoderma lucidum* gives some opportunity to discuss about

the growth characteristic as well as the potentialities of the fungus in the field of medicine. From the experimental data, it is revealed that the fungus favoured acidic medium for the optimum growth of mycelia and production of antimicrobial compounds. The present result support the reports of Hong *et al* (1981). The mycelial growth was found to be maximum at 30 day and antibiotic production started at 18 day after fermentation. Stark and Anke (1988) reported the production of antibiotic from submerged mycelia of *Lentinellus* sp during 13 day of fermentation. The optimum day for the maximum production of antibiotic way found to be different by a number of workers (Weber *et al., 1990; Anke et al.,* 1990; Laur *et al.,* 1991). The data on the role of terpenoids and polysaccharide on the growth bacteria revealed that these compounds were good enough to check the growth of a number of bacterial strains. Similar observations were supported by Stark and Anne (1988), Weber *et al* (1990), Anke *et al* (1990). *Lieu et al.* (1993). reported that *Ganoderma lucidum* (Curt; Fr.) is a herbal medicine with tumoricidal activity and they pointed out the vast potentialities of utilizing the bioactive compounds of this fungus for human welfare.

Table 2 : Antibacterial activity of the terpenoids and polysaccharides from mycelia of *Ganoderma lucidum*

Test organism[a]	Diameter of inhibition zone (mm)[b]	
	Terpenoids[c]	Polysaccharides[c]
Aerobacter aerogenes	26 ± 1.4	18±1.2
Bacillus brevis	36 ± 1.6	20±1.4
Bacillus subtilis	32 ± 1.6	22±1.4
Corynebacterium insidiosum	16 ± 1.0	18±1.2
Proteus vulgaris	26 ± 1.4	18±1.3
Clostridium pasteurianum	20 ± 1.2	20±1.4
Mycobacterium phlei	–	–
Sarcina lutea	–	–
Staphylococcus aureus	20 ± 1.2	16±1.4

REFERENCES

Anke, Timm and Werle, Andreas 1990. Antibiotics from Basidiomycetes XXXIII. Oudemanism X, a new antigungal E-Methoxyacry later from *Oudemansiella radicata* (Relhan ex Fr.) Sing *J.Antibiot,* **43 :** 1010-1011.

Anthony Manoni Mshandata and Jehova R. Mgonja. 2009. Submerged liquid fermentation of Tanzanian Basidiorycetes for theproduction of mycelial biomass, exopolysaccharide and mycetial protein using wastes peels media. ARPN Journal of Agricultural and Biological Science Nov., 2009.

Chairul, Takashi Tokuyama; Hycashi, Yoshinori; Nishizawa, Mugio;Toduka, Harukuni; Chairul, Sofrim and Hayashi Yuji 1991. Applanoxidic acid A.B.C and D biologically active tetracyclic triterpenses from *Ganoderma applanatum, Phytochemistry* (Oxf.) **30 :** 4105-4110.

Hassan, F.R.H., Ghada, M., Medany and El-Kady, A.T.M. 2012. Mycelial biomass production of Enoke Mushroom (Flammulina velutipes) by submerged culture. *Australlian Journal pf Basic and Applied science* **6 :** 603-610.

Hong, Jaesik; Kap, Sang Lee and Dong, Sung Choi 1981. Studies on basidimycets:1. Mycelial growth of *Agaricus bitroquis* and *Pleurotus ostreatus., Korean J. Mycol* **9:** 19-24

Kim, S.W., Hwang, H.J., Park, J.P., Cho Y.J., song, C.H.and Yun, J.W. 2002. Mycelial growth and exobiopolymer production by submerged culture of various edible mushrooms under different media. *Lett. Appl. microbial* **34:** 56-61.

Kwon, J.S., Lee, J.S. Shin, W.C.Lee, K.E.and Hong, E.K. 2009. Optimization of culture conditions and medium components for the production of mycelial biomass and exopolysaccharide with *Cordyceps* sp in liquid culture. *Biotechnol Bioprocess Eng* **14:** 756-762.

Laur, Ursula; Anke, Timm; and Hanssue, Fritz 1991. Antibiotics from *Lentinus adherens. J. Antibiot. (Tokyo)* **44:** 59'-65.

Lieu, Chien–Whei; Lee, Shiuh-Sheng and Wang, Sheng–Yuan 1993. The effect of *Ganoderma lucidum* on induction of differentiation of Leukemia U937 Cells - *Anticancer,* Res. **12:** 1211– 1215.

Lilly, V.G. and Burnett, H.L.1951 *Physiology of the fungi.* Mc. Graw Hill Book Co. London. 427.

Mizuno, Takashi, Ando, Motoharu; Sugie, Reiko; Ito, Hitoshi; Shimura, Keishiro; sumiya, Toshimitsu and Matsuura, Akira 1992. Anti tumor activity of some polysaccharide isolated from *Polyporus confluens, Biosci, Biotechnol, Biochem.* **56 :** 34-41.

Shiao, Ming Shi; Tseng, Tsung-Che; Hao, Yun-Yun and Shieh, Yuh-Shyan. 1986. Studies on *Ganoderma lucidum II.* The effect of Ganoderma lucidum on lipid metabolism in rats. *Bot. Bull. Acad. Sin.* (Taipei) **27:** 139-146.

Stark, A. and Anke,T. 1988. Lentinellic Acid, A Biologically active protoilludane derivative from *Lantinellus species* (Basidomycetes) (1). *Z. natur-forsch.* **43:** 177-183.

Wagner, R.,Mitch ell, D.A., Sassaki, G.L., Lopez del Almeida, Amazonas, M.A. and Berovi, M. 2003. Current techniques for the cultivation of *Ganoderma sp* for the production of biomass, ganoderic acid polysaccharides. *Food. Technol. Biotechno* **41:** 371-382.

Wang, Guanying; Zhang, Jie; Mizuno, Takashi; Zhuang, cun; ito, Hitoshi; Mayuzumi, Humimarcc, Okamoto, Hidehumi and Li, Jingzuan 1993. Antitumour active polysaccharides from the Chienese mushrom Songshan Lingzhi, the fruiting body of *Ganoderma tsugae. Biosic, Biotech. Biochem* **. 57:** 894-900.

Weber, Wolfgong; Anke, Timm; Stefan, Bewrt; Steglich, Wolfgang 1990. Antibiotics from basidiomycles XXXII. strobilurian–E. A new cytostatic and antifungal (E) – methoxy acrylate antibiotic from *Crepidotus fulvotomentosus Peek. J. Antiboot.* (Tokyo) **43:** 207-212.

Zhuang, Cun; Takashi, Mizuno; Shimada, Atsushi; ItoHitoshi; Suzuki, Chiharu; Mayuzumi, Yoshikazu; Okemoto, Hidehum; Ma, Yan and Li, Jingxuan 1993. Antitumor Protein containing polysaccharides from a chinese mushroom Fengweigu or Houbitake. Pleurotus sajor kaju (Fr.) Sings. *Biosci. Biotechnol Biochem* **57:** 901-906.