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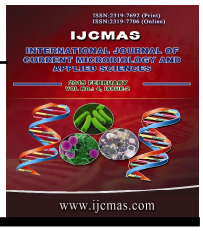

Original Research Article

Optimization of Sub-Merged Culture Conditions for Biomass Production in *Schizophyllum commune*, a Medicinal Mushroom

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Keywords

Schizophyllum commune, Temperature, pH, Agitation speed, Carbon and nitrogen sources

A B S T R A C T

In present study, the aim was to investigate the effect of physical factors such as temperature, pH, agitation speed, chemical compounds such as carbon and nitrogen sources, inoculum sizes and various media compositions on growth of *Schizophyllum commune*, a medicinal mushroom, were investigated. This fungus was able to grow at a temperature range of 10–40°C and pH range of 3.5–6.5. Optimal temperature and pH for the fungal growth was observed to be 25°C and 5.5 respectively. Exogenous supply of carbon or nitrogen source significantly enhanced the growth of the fungus. Among the carbon sources glucose (4%) was found to be the most stimulatory while molasses was least effective. In the same way, gram powder (4%) was found to be highly effective and ammonium nitrate was least effective as a nitrogen source. Fifteen mL of fungal biomass inoculum yielded 15.46g/L of end product. Highest growth rate of the test fungi was acquired on PDA + glucose medium after 15 days of incubation period. Finally it is concluded that optimization of submerged culture conditions for *Schizophyllum commune* will yield the maximum biomass production which can be used as unlimited sources of therapeutically useful biologically active agents.

Introduction

Mushrooms are a ubiquitous group of fungi with many uses. They reduce agricultural solid wastes through efficient biodegradation process furthermore they have many medicinal applications (Mugdha *et al.*, 2010).

They have effective substances for antifungal, anti-inflammatory, antitumor, antiviral, antibacterial, hepatoprotective, antidiabetic, hypolipedemic, antithrombotic and hypotensive activities (Wasser et al., 2000). In addition, they have high economic value because of their edibility and the enzymes they produce are very important for both basic research purposes and industrial applications (Ullrich *et al.*, 2004).

Schizophyllum commune is an edible mushroom having medicinal value and belongs to the phylum Basidiomycetes, order *agricales* family *Schizophllaceae*. Its

common name is split gill. The gills, which produce basidiospores on their surface split when the mushroom dries out (Alexopoulos *et al.*, 1996). It looks like a mini Oyster mushroom with one-fifth the size that of Fruiting bodies are produced each year; stalk is usually absent or very short. Fruiting can be solitary or in clusters on decaying hardwoods throughout the world. This fungus produces enzymes to decay the lignin in the wood causing "white rot". This is because of the cellulose left behind on the decaying wood. The fungus usually grows abundantly during the rainy season and frequently appears on dead woods (Zoberi, 1978). Schizophyllan is medically important polysaccharide extracted from *S. commune* having anticancer and anti-tumor activities (Ajith and Janardhanan, 2007).

Schizophyllum commune is a very good source of protein, vitamins, lipids and mineral elements. Other mushrooms like *Volvariella esculenta*, *Psathyereilla atroumbonata*, *Pleurotus* spp. and *Lentinus subnudus* are valued as food within the world (Jonathan and Fasidi, 2003). All these fungi are seasonal; they do not grow all year round under natural conditions. A range of a biotic parameters including temperature, light, carbon dioxide concentration, humidity, agitation speed and pH have been shown to influence mycelial growth and carpophore production (Wessels *et al.*, 1987). Therefore, mushrooms are commercially cultivated under controlled conditions.

Optimization of industrial mushroom production depends on improving the growth culture conditions (Larraya *et al.*, 2003). Hence, the present study was conducted to optimize growth condition for *Schizophyllum commune* using indigenous resources with the aim of providing useful information related to its cultivation biotechnology in Pakistan.

Material and Method

The young fruiting bodies of *Schizophyllum commune* were collected from decaying wood of *Mangifera indica* at Changa Manga Forest of Pakistan. The mycelia of this fungus were obtained using a modified tissue culture method of Quimio *et al.*, (1978) and maintained on potato dextrose agar (PDA OXOID ENGLAND) medium supplemented with 0.5% peptone (Merck, Germany). Analytical grade chemicals from Merck Germany were used throughout present study.

Micelial growth medium as described by Kirk and Tein (1988) with slight modifications, containing $KH_2PO_4.7H_2O$ (2.1 g), Mg $SO_4.7H_2O$ (0.3g), $CaCl_2$ (0.4g), $(NH_4)_2SO_4$ (2.0g), yeast extract (4g) malt extract (4g) and D-glucose (10g) peptone (5g) per 1000 mL of medium was used throughout the study or any change mentioned where required. The pH of the media was adjusted as per requirement of the experiment using 0.1N HCl/NaOH (pH meter ICM model 41100) for selection of optimal pH for fungal growth. Liquid medium (100mL) was dispensed into 250 mL flasks and 10 mg/100 mL of streptomycin (SIGMA, USA) added to suppress bacteria growth.

The flasks were covered with aluminum foil, autoclaved at 121±2°C under 15psi for 20 min. allowed to stand overnight in all cases. Fungal inoculum of 7 mm diameter disc of actively growing 5 days old mycelia of the mushroom was used in each experiment. The flasks were incubated at 25 $\pm$ 2^{o}C and observations were taken after incubation period of fifteen days. The mycelia produced were harvested by filtration through pre weight filter papers. Fungal dry

weight was determined accordingly by following mycelia dry weight method (Naseem *et al.*, 2001). Each treatment was replicated thrice and average results were recorded.

Optimum temperature, culture agitation speed, carbon/nitrogen source, inoculum size for mycelia growth of *S. commune* was determined at a pH of 5.5. Advantageous growth temperature was selected among 15, 25, 30, 35 and 40°C separately. To optimize the culture agitation speed 50, 75, 100, 150 and 200rpm was tried.

Effect of carbon and nitrogen sources on growth of *S. commune* was studied replacing said sources with 1, 2, 4 or 6% of glucose, sucrose, gram powder (chick pea) or ammonium sulphate individually in Kirk and Tein (1988) media. The medium without any carbon/nitrogen source served as the control. The optimal pH for this fungus growth was determined at pH 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5. After the set incubation period mycelia dry weight and pH of culture filtrate were determined from three replicates and the average recorded.

Three synthetic media, i.e. potato dextrose agar (PDA) (Oxoid, England), Malt Yeast Extract agar (MYEA) (Oxoid, England), Kirk agar (Merck, Germany) along with different combinations of carbon sources (shown in Table 1 or Fig. 4) were evaluated to determine their effect on linear mycelium growth of *S. commune* (expressed in cm/day).

An aqueous homogenate of *S. commune* mycelia, which was maintained on yeast extract enriched agar plates grown to the size of 50-70 mm in diameter, was prepared**.** Different concentration of *S. commune* homogenate, (5, 10, 15 and 20 mL) were then inoculated into 250mL conical flasks containing 100mL of basal medium. After 15 days mycelia were harvested, oven dried at 80^{o}C for 2 days and used for observations.

Statistical analysis

Mean values of biomass yield obtained from these studies were analyzed by one way analysis of variance (ANOVA) and test of significance difference were determined by Duncan's multiple range test (Snedeoor and Cochran, 1987).

Result and Discussion

Figure 1 depicts that the quantity of *S. commune* biomass produced is controlled by the pH of the nutrient growth media ranging from 3.5 to 6.5. The optimum pH for growth of *S. commune* was found to be 5.5 exhibiting highest vegetative growth of 12.19gL^{-1}. Lower growth of 10.75gL^{-1} and 10.63gL^{-1} as compared to the optimal growth (12.19gL^{-1}) was recorded at pH 5.0 and pH 6.0 respectively while the lowest growth (4.05, 4.09 and 6.85gL^{-1}) was observed, in the acidic media of pH 3.5, 4.0 and 4.5 respectively.

The results obtained in present study revealed that the *S. commune* produced biomass between temperatures of 10^{o}C to 40^{o}C. Among all temperatures tried for fungal growth maximum biomass (11.79gL^{-1}) was observed at 25^{o}C as shown in Figure 2. There was a considerable growth at 30^{o}C (9.91gL^{-1}), whereas growth at 10^{o}C and 40^{o}C was found to be 3.15gL^{-1} and 1.13gL^{-1} respectively as depicted in Figure 2.

In case of agitation speed, maximum mycelial growth (12.77gL^{-1}) of tested fungi was recorded at 150rpm. Whereas the fungal growth decreased markedly below and above the optimum stirring rate as evident from Figure 3.

During present investigation it was observed that supplementation of various carbon sources affected mycelial growth of *S. commune* significantly ($p<0.05$). It was recorded during this study that 4% concentration of each carbon source was best among all levels of supplementation (1-6%) for the fungus growth; however, 2 and 6% supplementation of all three carbon sources (glucose, sucrose, molasses) showed comparable growth to level of 4% as depicted in Figure 4. Glucose supported highest mycelia growth ($16.25gL^{-1}$) after 15 days of incubation (Figure 4). This was followed by sucrose ($15.65gL^{-1}$) and molasses ($8.99gL^{-1}$). Glucose and sucrose were comparable carbon sources while molasses having significantly lesser support for the growth of *S. commune*.

Effect of nitrogen on growth of *S. commune* was evaluated by changing the concentration of organic/inorganic nitrogen source within the range of 1 to 6%. Results revealed that maximum biomass production of test fungi in the presence of different nitrogen sources was at 4% concentration in each case. It was noticed that organic and inorganic nitrogen compounds supported moderate biomass production. The best biomass yield ($14.88gL^{-1}$) was found with gram powder closely followed by ammonium sulphate ($13.73gL^{-1}$), and ammonium nitrate ($10.97gL^{-1}$) as depicted in Figure 5.

S. commune was grown on PDA + glucose, PDA + sucrose, MYEA + glucose, MYEA + sucrose, Kirk agar media + glucose, Kirk agar media + sucrose and Kirk agar media + molasses to assess their growth rate. Maximum growth rate of test fungi was acquired on PDA + sucrose medium followed by PDA + glucose, MYEA + sucrose, MYEA + glucose after 15 days of incubation period. Kirk agar medium supplied with sucrose, glucose or molasses was found to be inferior to PDA /MYEA for supporting the fungal growth during present study as shown in Figure 6. PDA + glucose medium were observed to be significantly superior to all media composition tried.

Data presented in Tables 1 and 2 shows the effect of inoculum concentration on the growth of *S. commune* in different growth media. The biomass yield varied significantly ($P\leq0.05$) provided with 5–15mL of microbial inoculums while observed after 15 days of incubation period. Potato dextrose broth (PD) in combination with glucose was found to be the most favorable growth medium for *S. commune* at all microbial inoculum sizes ranging from 5mL to 15 mL as shown in Tables 1 and 2, however 15mL microbial inoculum produced maximum biomass yield of 1.602g/100mL.

MYE broth supplied with glucose was found to be after PDA + glucose broth with respect to microbial biomass production at all levels of inoculum size. Kirk medium combined with glucose or molasses at all inoculum sizes tried was not found to be favorable for fungal growth as shown in Table 1.

Present study revealed that pH 5.5 is optimal while a range of 3.5–6.5 was found to be suitable for the growth of *S. commune*. These results are not in accordance with the findings reported by Akinyele and Adetuyi (2005) and Kuforiji and Fasidi (1998). They reported the suitable pH range of 5.5 – 8.5 for *V. volvacea* and 5.0-7.0 for *Pleurotus tuberregium* respectively. These results showed that mushrooms require slightly acidic pH for maximum mycelial growth; while highly acidic or basic conditions could be destructive for the growth as stated by Kuforiji and Fasidi (1998).

Reusser *et al.* (1958) obtained similar results

with *Morchella hybrid* and *Trichoderma nudum*, they obtained maximum yield of mycelia at pH 5.0–5.5. Similarly present findings are in accordance with the reported results of Adejoye *et al.* (2007) they found greatest yield of *S. commune* at pH 5.5.

Temperature is an important environmental factor that controls the growth of most microorganisms. It was found in present study that 25°C is the most favorable temperature for the growth of *S. commune* which is supported by the results of Adejoye *et al.* (2007), they reported *S. commune* had its optimum growth at 25°C with mycelial extension of 102.97 mm. Present results are in contradiction to Akinyele and Adetuyi (2005) who recorded 30°C to be most advantageous temperature for *V. volvacea.*

The Agitation speed of tested fungi was maximum at 150 rpm whereas, the fungal growth decreased markedly below and above the optimum stirring rate. Our results are in accordance to Tomokazu and Noriyasu (2001) who reported the significance of agitation speed in submerged fermentation on the growth of mushrooms.

Glucose and sucrose have been reported as good energy sources for cellular vegetative growth (Hammonod, 1978). Likewise, Kim *et al.* (2005) reported the preference of sucrose as best supplement for growth of ascomycetous fungi; however in other results Fang-Hong et al. (2001) reported glucose as best option for *Polyporus umbellatus*. Reusser *et al.* (1958) obtained similar results with *Morchella hybrid* and *Trichoderma nudum*, they found maximum yield of mycelia at 4–6% glucose supplied in the media.

Likewise, Kim *et al.* (2005) reported the preference of sucrose as best supplement for growth of ascomycetous fungi; however in other results Fang-Hong et al. (2001) reported glucose as best option for *Polyporus umbellatus*.

Present study showed that natural source of nitrogen that is gram powder is the most suitable for *S. commune* as compared to chemical sources of nitrogen i.e. ammonium sulphate and ammonium nitrate. These results are not in corroboration with Gbolagade *et al.*, (2006) who have reported that *Pleurotus florida* (mont.) Singer yielded highest quantity of biomass while provided with ammonium nitrate which was closely followed by potassium nitrate. Jonathan and Fasidi (2001) have also reported contrary results for *P. atroumbonata*.

Present Data showed that PDA as a semi solid media was most suitable media for both rate and amount of fungal growth. The results are in agreement with the observations reported by Nasim *et al.*, (2001) about growth *Pleurotus ostre*atus. During present study *S. commune* growth profile showed that an inoculum of 15mL was favorable for higher biomass production which is contrary to the findings of Jin-Zhong et al. (2003) for *P. tuber-regium*.

This piece of research has shown that noteworthy enhancement in the mycelia growth of S. commune, having value for its high nutritional composition can be attained through cultivation of the fungus on pH 5.5 and temperature of 25°C. Potato dextrose agar supplied with glucose as a carbon source promoted good vegetative growth and gram powder was the best utilizable nitrogen source most suitable for its cultivation.

This result may provide a sustainable means of adding importance to *S. commune* cultivation which will result in increasing human dietary protein. Therefore, we

conclude that appropriate harvest time, pH, temperature, inorganic and organic compounds and inoculums size are critical factors in optimizing biomass yield of *S. commune.*

Fig.1 Effect of Temperature

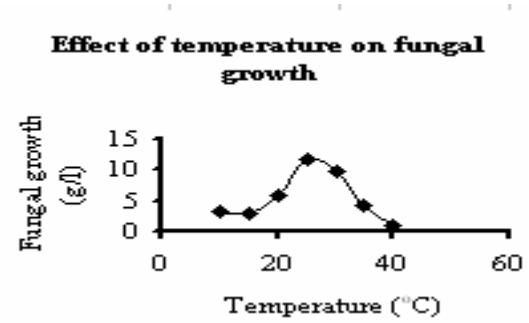

Fig.2 Effect of pH

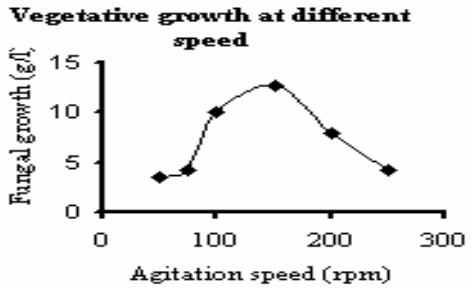

Fig.3 Effect of agitation speed

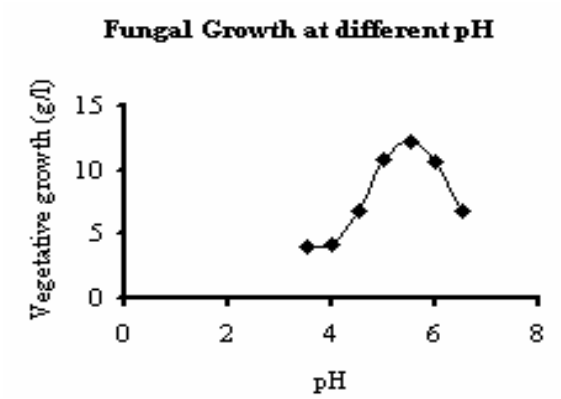

Fig.4 Effect of carbon sources

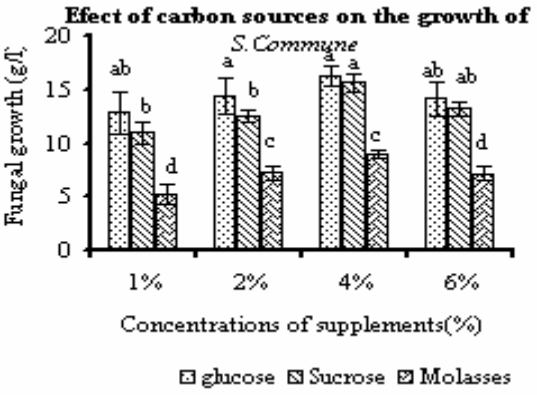

Fig.5 Effect of Nitrogen sources

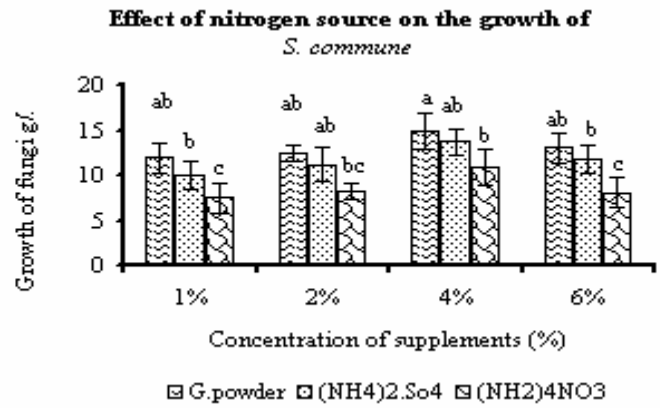

Fig.6 Effect of different media on *S. commune* growth

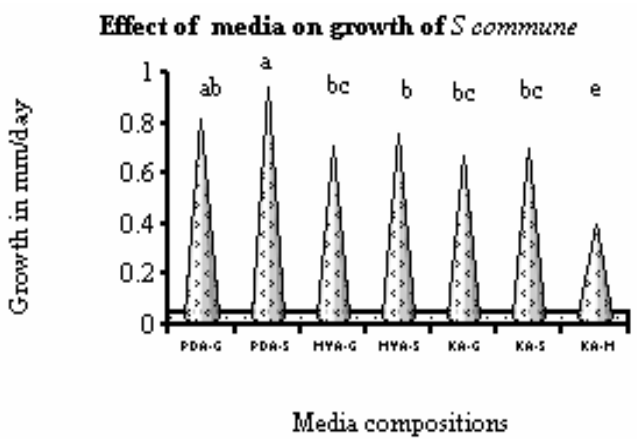

Table.1 Effect of different media on *S. commune* growth

Medium	Inoculum (ml/100ml)	3rd days	6th days	9th days	12th days	15th days
PD+ glucose	5 ml	0.066	0.264	0.383	0.459	1.201
MYE+ glucose		0.052	0.127	0.258	0.383	1.063
Kirk+ glucose		0.044	0.084	0.177	0.272	0.812
Kirk+Molasses		0.039	0.048	0.137	0.228	0.646
PD+ glucose	10ml	0.221	0.291	0.465	0.599	1.244
MYE+ glucose		0.184	0.199	0.364	0.502	0.971
Kirk+ glucose		0.206	0.281	0.304	0.444	0.744
Kirk+Molasses		0.161	0.192	0.274	0.399	0.577
PD+ glucose	15 ml	0.578	0.633	0.707	1.298	1.602
MYE+ glucose		0.171	0.194	0.244	0.426	0.739
Kirk+ glucose		0.165	0.249	0.271	0.367	0.654
Kirk+Molasses		0.127	0.181	0.252	0.27	0.496
PD+ glucose	20 ml	0.479	0.514	0.658	1.019	1.12
MYE+ glucose		0.164	0.156	0.166	0.382	0.605
Kirk+ glucose		0.165	0.233	0.249	0.308	0.572
Kirk+Molasses		0.114	0.149	0.241	0.265	0.388

Table.2 Effect of inoculum sizes on the growth of *S. commune*.

Medium	Inoculum (ml/100ml)	Mean values growth g / 100ml				
		3rd day	6th day	9th day	12th day	15th day
PD+ glucose	5 ml	0.046	0.264	0.183	0.459	1.201
MYE+ glucose		0.052	0.127	0.158	0.383	1.063
Kirk+ glucose		0.044	0.084	0.147	0.342	0.812
Kirk+Molasses		0.039	0.048	0.137	0.328	0.646
PD+ glucose	10ml	0.221	0.241	0.265	0.599	1.244
MYE+ glucose		0.184	0.199	0.224	0.522	0.971
Kirk+ glucose		0.206	0.381	0.444	0.444	0.844
Kirk+Molasses		0.201	0.452	0.374	0.399	0.677
PD+ glucose	15 ml	0.578	0.633	0.707	1.298	1.602
MYE+ glucose		0.491	0.594	0.744	0.826	1.439
Kirk+ glucose		0.385	0.449	0.571	0.767	0.754
Kirk+Molasses		0.327	0.391	0.552	0.700	0.696
PD+ glucose	20 ml	0.479	0.614	0.658	1.309	1.548
MYE+ glucose		0.304	0.456	0.566	0.382	0.805
Kirk+ glucose		0.265	0.363	0.349	0.408	0.772
Kirk+Molasses		0.194	0.349	0.541	0.575	0.588

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