

Original Research Article

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Screening of Different *Schizophyllum commune* strains on Different Culture Media and Different Temperatures

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Present study was carried out to standardize culture media and temperature conditions for optimal growth of *Schizophyllum commune* (split-gill mushroom) strains. Radial growth of six strains was evaluated on PDA, MEA and other substrate extracts media at $20\pm1^\circ\text{C}$, $25\pm1^\circ\text{C}$ and $30\pm1^\circ\text{C}$ temperatures at 7 and 14 days after inoculation. Strain SC-06p exhibited maximum radial growth of 34.66 mm on PDA media and 32.00 mm on MEA media at 7 days post-inoculation and at 14 days after inoculation. SC-06p strain showed maximum radial growth of 58.33 mm on PDA media followed on MEA media of 51.11 mm radial growth. Optimal temperature for SC-06p was observed at $30\pm1^\circ\text{C}$ yielding 34.66 mm and 31.00 mm by strain SC-06p at $25\pm1^\circ\text{C}$ at 7 days after inoculation. Strain SC-06p gave maximum radial growth of 58.33 mm radial growth at $30\pm1^\circ\text{C}$ and 52.33 mm radial growth at $25\pm1^\circ\text{C}$ at 14 days post-inoculation.

Introduction

Mushrooms are one of the first forms of microbial food known to mankind. About 1.5 million types of fungi are existing, but only 14,000 mushroom types have been discovered (Hawksworth, 2001) of which, well-studied mushroom species are less in number. Mushrooms belong to the class Basidiomycetes, they are spore-bearing fruiting bodies which grow above ground on soil or on other food sources. Only 700 species of mushroom are edible and 50 species are toxic. These are high in fibre, low in fat, contains all critical minerals and amino acids except iron (Sadler, 2003). Basidiomycetes, which

include medicinal mushrooms, are rich source having a wide range of biological mushrooms, but they are mostly untapped. Around 650 mushrooms have medicinal properties that are similar to edible species like *Hericium erinaceus*, *Lentinus edodes*, *Grifola frondosa*, *Flammulina velutipes*, and *Tremella spp.* In general, edible mushrooms are high in vitamin B, D, K and rarely in A and C but are low in fat and calories (Alam *et al.*, 2007), these are good source of minerals and contain more protein than any other plant-based diet (Qin, 1989), hence mushrooms provide a valuable dietary supplement.

Schizophyllum commune, belongs to the Phylum Basidiomycota, Sub-phylum Agaricomycotina, Order Agaricales, Family Schizophyllaceae and genus *Schizophyllum*. The distinguishing feature of these fungi is the production of a macroscopic fruitification, the basidiocarp, that includes basidiospores (sexual spore) forming on the outside of club-shaped or elongate structure, the basidium. It is an edible mushroom with a range of medicinal properties, found growing on dead and decaying organic waste, mainly on rotten tree wood. It is grown in all continents except Antarctica, as there is no wood found as a substrate (Khatua *et al.*, 2013). *S. commune* is also known as “Split gill” which gets its name from the “gills” on the underside of cap that are divided longitudinally. This mushroom has the appearance of undulating coral waves or a loose Chinese fan. The colour of “gillies” or “split gills” ranges from creamy yellow to pale white. The cap is tiny with solid yet spongy body texture and a width of 1-4 centimetres (388-588 in.) (Kuo, M. *et al.*, 2003). The crowns are 1-4 centimetres (30.8-1 + 50.8 in.) broad with white or greyish hairs. They grow in shelf-like patterns without stalks (Davis *et al.*, 2012). After drying, the gills which generate basidiospores on their surface, split, giving the fungus the common name split gill (Guarro *et al.*, 1999). The mushrooms can be dried for decades before being resurrected with wetness (Davis *et al.*, 2012). *S. commune* consumed globally as food, medicine and a bioremediation agent. It is highly rich in protein, vitamin, fat and mineral content (Adejoye *et al.*, 2007). It has high dietary fibre content of more than 50% of the net weight and is high in P, Mg, K and Se (Ghorai *et al.*, 2009). Extracts of *S. commune* plant have antibacterial and antifungal activities, making them suitable antimicrobial agents.

Materials and Methods

Five strains of *Schizophyllum commune* viz., SC-01, SC-02, SC-03, SC-04, SC-05 were collected from DMR Solan and SC-06 is collected from Dr. Rajendra Prasad Central Agricultural University's Advance Centre of Mushroom Research CBS&H, Department of Microbiology, Pusa, Samastipur, Bihar. The trials were conducted at Dr. Rajendra Prasad Central Agricultural University's Advance Centre of Mushroom Research, CBS&H, Department of Microbiology, Pusa, Samastipur, Bihar.

Preparation of Culture Media

Potato Dextrose Agar (PDA) media, Malt Extracts Agar

(MEA) media and Substrate extracts media like Sawdust extract, Wheat straw extract, Paddy straw extract, maize straw extract, Litchi leaf extract, tea waste extract were used during laboratory studies on *Schizophyllum commune*.

Preparation of Potato Dextrose Agar medium (PDA)

Boil 200g of peeled and sliced potatoes in 500ml of distilled water until soft, then filter the extract through a cotton cloth. Add 20g dextrose and 20g agar to the filtrate, stir thoroughly, and boil the mixture for 15 min. Mix the dissolved agar to dextrose-potato extract and make 1 litre volume and dispense 200ml aliquots into five conical flasks plugged with non-absorbent cotton wool, sterilize in an autoclave, and pour plates aseptically into petri dishes.

Preparation of Malt Extract Agar medium

Dissolve 20g of malt extract in 500ml distilled water, add 20g of Dextrose to the malt extract solution and thoroughly stir it. Simultaneously, 20g of agar are mixed with 6g of peptone in a beaker holding 500ml of distilled water and heated for 15 minutes. The malt extract solution is completely soluble after boiling, resulting in a volume of 1 litre. Dispense 100ml aliquots into five conical flasks plugged with non-absorbent cotton wool and sterilize by autoclaving prior to use in petri dishes.

Preparation of substrate extracts media

Sawdust, Wheat straw, Paddy straw, Maize straw, Litchi leaf and Tea waste 20g each are all cleaned under running water before cooking for 20 minutes separately in 1 litre of distilled water and filtered. 20g Dextrose and 20g agar were added to each medium and the solution was stirred until the agar completely melts on a low flame resulting in 1 litre of volume and fill in the conical flasks (Table 1). After filling, plug the conical flasks using non-absorbent cotton plugs and autoclave.

Effect of different media on radial growth of *Schizophyllum commune*

Testing of six strains of *Schizophyllum commune* were done on eight different media, including Potato dextrose agar, Malt extract agar, Sawdust extract, Wheat straw extract, Paddy straw extract, Maize straw extract, Litchi leaf extract and Tea waste extract medium. 25ml of the

appropriate medium was poured in the sterile petri plates, a little quantity of streptomycin was added to each medium to avoid any contamination. After solidification of medium, 7 days old culture of different strains is inoculated at the centre of petri plates. For each

treatment, the inoculated plates were kept at $30\pm1^{\circ}\text{C}$ and replicated three times. Observations were made in the form of radial growth at 7- and 14-days following inoculation.

Table.1 Substrate extracts media used during laboratory screening of *Schizophyllum commune*

S. No.	Substrate Extracts Media	Composition
1	Sawdust Extract Medium	
	Sawdust Dextrose Agar-agar Distilled water	20 g 20 g 20 g 1000 ml
2	Wheat straw Extract Medium	
	Wheat straw Dextrose Agar-agar Distilled water	20 g 20 g 20 g 1000 ml
3	Paddy straw Extract Medium	
	Paddy straw Dextrose Agar-agar Distilled water	20 g 20 g 20 g 1000 ml
4	Maize straw Extract Medium	
	Maize straw Dextrose Agar-agar Distilled water	20 g 20 g 20 g 1000 ml
5	Litchi leaf Extract Medium	
	Litchi leaf Dextrose Agar-agar Distilled water	20 g 20 g 20 g 1000 ml
6	Tea waste Extract Medium	
	Tea waste Dextrose Agar-agar Distilled water	20 g 20 g 20 g 1000 ml

Effect of different temperature on radial growth of *Schizophyllum commune*

Effect of different temperature was done by inoculating each strain with an actively developing 7 days old pure culture in petri plates containing 25ml of Potato

Dextrose Agar (PDA) medium, each treatment with three replications. The inoculated plates were then

exposed to various temperatures: $20\pm1^{\circ}\text{C}$, $25\pm1^{\circ}\text{C}$ and $30\pm1^{\circ}\text{C}$ for incubation. At 7 and 14 days after inoculation, the observations were recorded as radial growth.

Statistical Analysis

All of the trials were evaluated using Randomized block design (RBD), and the data was statistically analysed

using the web programme OPSTAT. Standard error of mean (Sem) and critical difference (C.D) were determined at 5% level.

Results and Discussion

Effect of different media on radial growth of different *Schizophyllum commune* strains

The results showed that all media supported the mycelial growth of all strains. PDA media showed the best results of radial growth at 7 and 14 days after infection compared to all other media. After 7 days of infection, maximum radial growth was observed on PDA by strain SC-06p (34.66 mm), on MEA by strain SC-06p (32.00 mm), on WSE by strain SC-06p (27.66 mm), on TEA by strain SC-06p (29 mm), on SDE media by strain SC-06p (24.33 mm), on MSE by strain SC-06p (22.33 mm), on LLE by strain SC-06p (23.33 mm). Among all the media, poor growth was observed on PSE media in which maximum growth is observed in Strain SC-06p (22.66 mm). The minimum radial growth was observed by strain SC-02 (18.66 mm) on PDA medium, by strain Sc-04 (18.00 mm) on MEA media, by strain SC-03 (14.66 mm) on WSE media, by strain SC-02 (15.00 mm) on TEA media, by strain Sc-02 (12.66 mm) on SDE media, by strain SC-02 (12.66 mm) on MSE, by strain Sc-02 (11.33 mm) on LLE, by strain SC-02 (12.66 mm) on PSE media (Table 2).

After 14 days, the mycelium growth of different strains of *S. commune* fully covered the media surface and maximum radial growth was observed on the PDA medium in strain SC-06p (58.33 mm) whereas the minimum radial growth was observed in strain SC-02 (49.33 mm). Followed by MEA medium, maximum radial growth was shown by strain SC-06p (53.66 mm) and minimum by strain SC-02 (46.33 mm) was noticed. However, in substrate extract media the maximum radial growth observed in the TWE and WSE followed by SDE and MSE. The minimum radial growth was observed in LLE followed by PSE as presented in . On TWE after 14 days the maximum radial growth observed in strains SC-06p (51.66 mm), minimum in strain SC-04 (41.66 mm). The WSE medium showed the maximum radial growth in strains SC-06p (50.00 mm), minimum in strain SC-02 (41.66 mm). On SDE after 14 days, the maximum radial growth observed in strains SC-06p (47.33 mm) whereas minimum in strain SC-03 (41.33 mm). On MSE after 14 days, the maximum radial growth observed in strains SC-06p (42.66 mm), minimum in strain SC-02 (35.00 mm). On LLE after 14 days the maximum radial growth observed in strains SC-06p (40.66 mm) and minimum in strain SC-02 (34.00 mm). On PSE after 14 days, the maximum radial growth observed in strains SC-01 (37.66 mm) and minimum in strain SC-02 (32.66 mm). The PDA medium yielded significantly maximum radial growth and significantly superior to others.

Table.2 Effect of different media on radial growth of different *Schizophyllum commune* strains at 7 days.

Media	Radial growth of strains (mm)*						Mean
	SC-01	SC-02	SC-03	SC-04	SC-05	SC-06p	
PDA	29.33	18.66	24.66	25.33	28.66	34.66	26.89
MEA	28.33	24.00	22.66	21.66	23.66	32.00	25.39
SDE	22.33	12.66	14.33	16.66	17.33	24.33	17.94
WSE	24.00	16.33	14.66	18.00	19.66	27.66	20.06
PSE	19.33	12.66	11.33	15.00	15.33	22.66	16.06
MSE	19.66	12.66	13.33	16.66	16.33	22.33	16.83
LLE	19.33	11.33	14.00	15.33	16.00	23.33	16.56
TWE	26.33	15.00	16.66	18.66	21.00	29.00	21.11
MEAN	23.58	15.42	16.46	18.42	19.75	27.00	
Factors	C.D. (5%)			SE(d)	SE(m)		
Media	0.344			0.173	0.122		
Strains	0.298			0.150	0.106		
Media × Strains	0.843			0.424	0.300		

(*) – Average of three replications

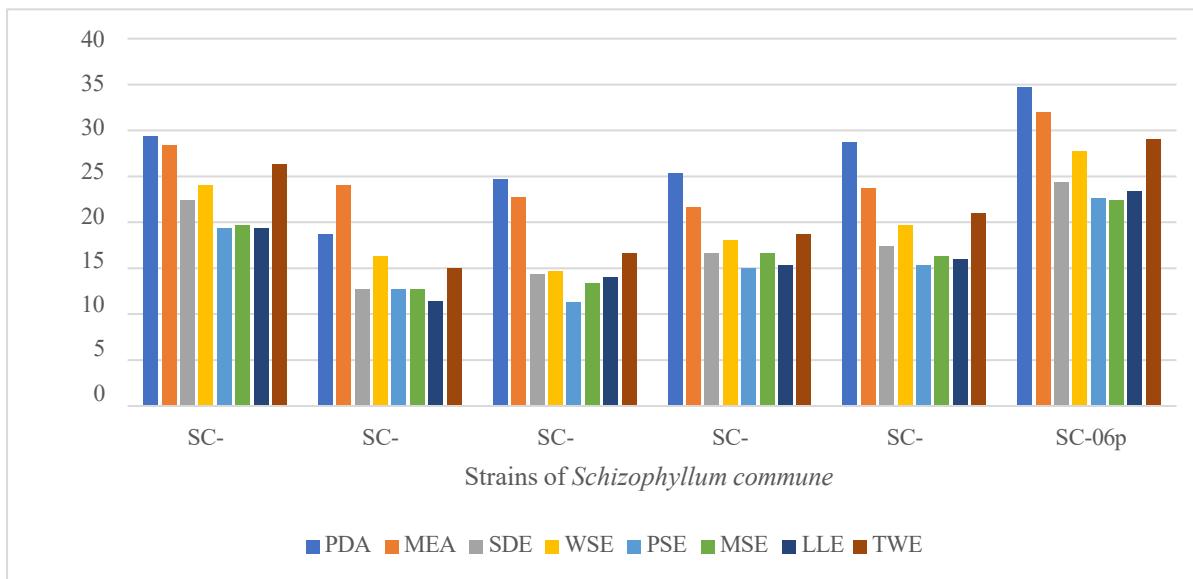
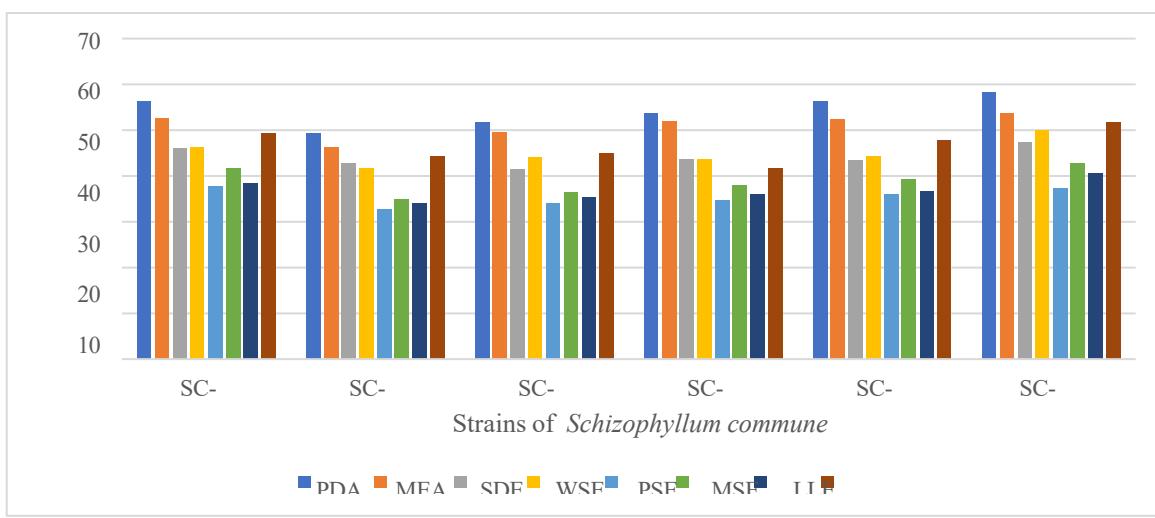


Table.3 Effect of different media on radial growth of different *Schizophyllum commune* strains at 14 days

MEDIA	Radial growth of strains (mm)*						MEAN
	SC-01	SC-02	SC-03	SC-04	SC-05	SC-06p	
PDA	56.33	49.33	51.66	53.66	56.33	58.33	54.28
MEA	52.66	46.33	49.66	52.00	52.33	53.66	51.11
SDE	46.00	42.66	41.33	43.66	43.33	47.33	44.06
WSE	46.33	41.66	44.00	43.66	44.33	50.00	45.00
PSE	37.66	32.66	34.00	34.66	36.00	37.33	35.39
MSE	41.66	35.00	36.33	38.00	39.33	42.66	38.83
LLE	38.33	34.00	35.33	36.00	36.66	40.66	36.83
TWE	49.33	44.33	45.00	41.66	47.66	51.66	46.61
MEAN	46.04	40.75	42.17	42.91	44.50	47.71	
Factors	C.D. (5%)		SE(d)		SE(m)		
Media	0.827		0.416		0.294		
Strains	0.716		0.360		0.255		
Media × Strains	2.026		1.019		0.720		



Comparing all the eight different media (PDA, MEA, SDE, WSE, PSE, MSE, LLE and TWE) in present study, PDA medium has shown maximum radial growth at both 7 and 14 days after inoculation. The present finding was close to the result obtained by Singh *et al.*, (2021) and Fitriyan *et al.*, (2020) who found that *Schizophyllum commune* grew well on the PDA media, maximum radial growth was recorded

Effect of different temperature on radial growth of different *Schizophyllum commune* strains.

Among different temperatures, the most favourable temperature was $30\pm1^{\circ}\text{C}$ for all strains of *S. commune* which gave maximum radial growth at 7 days i.e., 34.66 mm in strain SC-06p and minimum growth was recorded by strain SC-02 (18.66 mm). When the temperature was decreased from $30\pm1^{\circ}\text{C}$ to $20\pm1^{\circ}\text{C}$ the growth of all the strains decreased at 7 days i.e., 27.33 mm in strain SC-06p and minimum growth was recorded by strain SC-02 (14.66 mm). When the temperature was increased from $20\pm1^{\circ}\text{C}$ to $25\pm1^{\circ}\text{C}$ the growth of strains increased compared to $20\pm1^{\circ}\text{C}$ temperature at 7 days i.e., 31.00 mm in strain SC-06p and minimum growth was recorded by strain SC-02 (16.66 mm).

After 14 days at $30\pm1^{\circ}\text{C}$ temperature, all strains of *S. commune* produced maximum radial growth. The maximum radial growth was observed by strain SC-06p (58.33mm) and minimum radial growth was recorded by strain SC-02 (49.33 mm). At $25\pm1^{\circ}\text{C}$ temperature maximum radial growth was observed by strain SC-06p (52.33 mm) and minimum radial growth was recorded by strain SC-02 (46.33 mm). At $20\pm1^{\circ}\text{C}$ temperature maximum radial growth was observed by strain SC-06p (49.66 mm) and minimum radial growth was recorded by strain SC-02 (43.33 mm). A Clear difference was observed in the radial growth of different strains of *Schizophyllum commune* at different temperature range ($20\pm1^{\circ}\text{C}$, $25\pm1^{\circ}\text{C}$, $30\pm1^{\circ}\text{C}$) at 7 and 14 days. Among the three different temperatures ($20\pm1^{\circ}\text{C}$, $25\pm1^{\circ}\text{C}$, $30\pm1^{\circ}\text{C}$), $30\pm1^{\circ}\text{C}$ was found to be most suitable temperature for the mycelial growth of different strains of *Schizophyllum commune*. At $30\pm1^{\circ}\text{C}$ maximum radial growth was recorded at both 7 and 14 days. The present results obtained was corresponded with the results of Aminah *et al.*, (2020) who reported that mycelial growth was highest at 30°C temperature. Reddy *et al.*, (2017) studied on cultural and physiological studies on *Schizophyllum commune* and *Lentinula edodes* Wild Mushroom Specimens were done. The investigation found that

potato dextrose agar medium with a pH range of 5.0-7.0 at a temperature of 30°C and malt extract medium with a pH range of 4.5-6.5 at a temperature of 25°C were optimal for the mycelial growth of *S. commune* and *L. edodes*, respectively.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

Panchagiri Akhil: Investigation, analysis, writing original draft, Dayaram: Methodology, writing-reviewing, Birudukota Monika: Conceptualization, methodology, writing,

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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