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Research Article

Comparison of colony diameter, growth rate and biomass dry weight of fungal cultures on liquid and solid media

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Abstract An evaluation was carried out to analyze the growth performance and biomass production of *four standard fungal* cultures namely, *Coriolus versicolor* MTCC 138, *Phanerochaete chrysosporium* MTCC 787, *Pleurotus ostreatus* MTCC 142 and *Aspergillus oryzae* on three different agar media; Potato Dextrose agar medium (PDA), Yeast Glucose extract agar Medium (YGEA) and Malt Extract agar Medium (MEA) and broth media; Potato dextrose broth (PDB), Yeast glucose extract broth (YGEB) and Malt extract broth (MEB). On comparative analysis of higher mycelial growth and biomass production of the four test fungi, the best performance where observed in PDA and PDB. These results will find use in fungal taxonomic studies.

Keywords: Coriolus versicolor MTCC 138, Phanerochaete chrysosporium MTCC 787, Pleurotus ostreatus MTCC 142, Aspergillus oryzae, Media, colony diameter, biomass, growth rate.

Introduction

Culture or growth medium is generally defined as a substrate that contains all the elements required to support the growth of microorganisms or cells outside their natural habitat (Kumar and Bhadauria 2017) and preparation of a suitable culture medium is the pre-requisite to study them. The growth media along with the environmental factors play an important role in growth and reproduction of microorganisms (Kumaran and Jeya 2014).

Different microorganisms have different growth requirements like temperature, pH, moisture, and osmotic requirements (Basu *et al*.2015). Due to failure in replication of exact natural conditions, nearly 99% of all microorganisms fail to grow in laboratory conditions. Fungi are eukaryotic and heterotrophic organisms, which uses external sources of organic compounds for food. These grow as multicellular filaments called hyphae forming a mycelium; while some species also grow as single cells (Nwogu and Elenwo 2012). Specific media are used for isolating, cultivating, preserving fungi (Devi *et al*. 2018). They require media with high carbon and nitrogen content, with a pH ranging from 5-6 and optimum temperature from 15-40°C. Generally, two types of culture media are used for studying fungal growth namely natural and synthetic media.

Natural media are easy to prepare and are composed of natural substrates of unknown composition such as corn meal, leaves, potato dextrose agar etc. Synthetic media namely Czapek-Dox medium etc. is composed of known components and contains a defined amount of carbon, nitrogen and vitamins (Collins *et al*. 2005). Fungi can be directly utilized as a source of food (in the form of mushrooms and truffles) and in fermentation of various food products (soy sauce, wine, beer etc). Recently, these are used as sources of antibiotics and enzymes, such as cellulases, hemicellulases, ligninases and proteases (Nwogu and Elenwo 2012).

Materials and Methods

Standard fungal cultures

Four standard cultures namely *Pleurotus ostreatus*

MTCC 142, *Coriolus versicolor* MTCC 138, *Phanerochaete chrysosporium* MTCC 787 and *Aspergillus oryzae* were procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh.

Growth profile of fungal cultures

The growth profile of all the procured cultures namely *Pleurotus ostreatus* MTCC 142, *Coriolus versicolor* MTCC 138, *Phanerochaete chrysosporium* MTCC 787 and *Aspergillus oryzae* was studied on different media like yeast glucose extract media, malt extract media, and potato dextrose media under solid and liquid conditions. To study the effect on growth rate, sterilized agar plates of different media were prepared and inoculated with the 8mm mycelia bit of each fungal culture in the centre of the plate. The plates were incubated at their respective temperatures for 7 days and radial diameter was measured at an interval of two days. The growth rate was calculated as per formula given below.

$$\text{Growth rate (cm day}^{-1}) = \frac{\text{Colony diameter}}{\text{Incubation period}}$$

To study the effect of media on fungal biomass, Erlenmeyer flasks of 250ml capacity were dispensed with 100ml of different broth in triplicates and were sterilized in the autoclave at l21°C at 15 lbs pressure for 20 min. The flasks were inoculated with 3 mycelial bits (8mm diameter) of each culture separately and incubated at their respective temperatures (Table 1) for 20 days. The flasks were removed from the incubator after every 2 days till 20 days. The mycelial mats were filtered through a pre weighed Whatman No.1 filter paper and dried at 50°C for 24 hours. The dry weight of the mycelium was calculated as per the formula given below.

Dry weight (g) = W_2-W_1

where, W_1= weight of Whatman No.1 filter paper

W_2= weight of fungal mat + weight of Whatman No.1 filter paper after drying

Results and Discussion

Table 2 represents the effect of different agar media on the radial growth and growth rate of selected fungal cultures. The fungal cultures were inoculated on three different solid media for 6 days and growth was measured at an interval of two days. It was observed that all of them supported the growth of fungal cultures to various degrees. *P. ostreatus* MTCC 142 showed maximum growth on MEA with colony diameter of 7.4cm on 6th day and growth rate of 1.23cm day^{-1}, followed by PDA and YGEA with colony diameters of 6.8 cm and 6.2 cm and growth rates of 1.13cm day^{-1} and 1.03 cm day^{-1}, respectively.

In a similar study carried out by Mahadevan and Shanmugasundaram (2018) also evaluated the effect of six different media on the growth of *Pleurotus sapidus*. It was observed that Potato Dextrose Agar supported maximum growth with colony diameter of 8.97cm and growth rate of 0.9cm day^{-1} followed by Malt Extract Agar, and Yeast Malt Agar medium showed maximum growth rate and density.

Fletcher *et al* (2019) investigated the effect of four different media namely PDA, PDA supplemented with yeast extract, PDA supplemented with iron sulphite and sabouraud dextrose agar, on mycelial growth of *Pleurotus ostreatus* and *Ganoderma lucidum*. It was reported that the highest radial diameter of 23.28cm was observed in case of PDA followed by PDYA (14.73cm), while the mycelial growth was poor in SDA (9.85cm) and ISA (8.35cm). Singh and Singh (2018) studied the effect of six different media on the radial growth and growth rate of *Pleurotus djamor* and highest radial growth of 88.33mm and growth rate of 11.04 mm day^{-1} was observed in oat extract agar medium, followed by barley extract agar media with radial growth and growth of 83.00mm and 10.37mm, respectively.

Similarly, *C. versicolor* MTCC 138 showed maximum growth on PDA with colony diameter of 7.6cm on 6th day and growth rate of 1.26cm day^{-1}, followed by MEA and YGEA with colony diameters of 6.0cm and 4.6 cm and growth rates of 1.00cm day^{-1} and 0.77 cm day^{-1}, respectively. In a similar studies carried out by Jo *et al* (2010), in which the effect of media on five different species of *Coriolus* was evaluated, it was reported that PDA, MEA and YEA supported the maximum growth of *Coriolus,* while it was poor in glucose peptone, Czapek Dox and Hennerberg media.

The highest colony diameter of *P. chrysosporium* MTCC 787 was 7.2cm, observed in case of PDA, with growth rate of 1.20cm day^{-1}, followed by YGE with colony diameter of 6.4cm day^{-1} and growth rate of 1.06cm day^{-1}, after 6th day of incubation. However, MEA displayed slowest, with colony diameter of 4.6cm and growth rate of 0.76cm day^{-1}. The highest colony diameter of *A. oryzae* was 7.8cm, observed in case of PDA, with growth rate of 1.30cm day^{-1}, followed by MEA with

colony diameter of 7.0cm day^{-1}and growth rate of 1.17cm day^{-1}, after 6th day of incubation. However, YGEA displayed slowest growth *A. oryzae,* with colony diameter of 5.8cm and growth rate of 0.96cm day^{-1}.

To study the biomass production, fungal cultures were grown on Potato dextrose broth (PDB), Yeast glucose extract broth (YGEB) and Malt extract broth (MEB) and were incubated for 20 days. The dry weight was measured at an interval of two days, by filtering, drying and weighing the fungal mat in a dry filter paper. Table 3-5 represents dry biomass of fungal cultures on different broth media. Table 3 shows the growth profile of selected fungal cultures on PDB, in which *C. versicolor* MTCC 138 showed maximum dry weight of 2.52g, followed by *P. ostreatus* MTCC 142 (1.65g), *A. oryzae* (1.35g) and *P. chrysosporium* MTCC 787(1.09g) in that order, after 20 days of incubation.

Similarly, Table 4 shows the growth profile of fungal cultures on YGEB broth media where, *A. oryzae* showed maximum dry weight of 1.6g, followed by *P. ostreatus* MTCC 142 (1.17g), *C. versicolor* MTCC 138 (0.94g) and *P. chrysosporium* MTCC 787(0.78g) in that order, after 20 days of incubation. Table 5 represents the results of growth rate on MEB, in which, *C. versicolor* MTCC 138 showed maximum dry weight of 1.98g, followed by *A. oryzae* (1.49g), *P. ostreatus* MTCC 142 (1.23g) and *P. chrysosporium* MTCC 787(0.98g) in that order, after 20 days of incubation. It was concluded from results that *C. versicolor* MTCC 138 was the fastest growing fungi among the four selected cultures followed by *A. oryzae, P. ostreatus* MTCC 142 and *P. chrysosporium* MTCC 787.

Rawte and Diwan (2011) studied the biomass production of *P. florida. P. sajor-caju, P. ostreatus* and *P. flabellatus* for 9 days in various broth media and reported highest biomass production on Potato dextrose media inculated with with *P. florida* (1.86 g). Similarly, Nwogu and Elenwo (2012) evaluated Soybean and groundnut dextrose broth (GDB), potato dextrose broth (PDB) and sawdust sucrose broth (SSB) for the cultivation of *Aspergillus niger, A. flavus, A. terreus, A. glaucus, Penicillium chrysogenum, Fusarium oxysporium,* and *Rhizopus stolonifer.* After incubating for 7 days at 27 ± 1°C, the cultures were harvested and weighed to study the growth rate. It was found that the growth rate was maximum in SSB, because it has more vitamins and minerals compared to other media, followed by GDB and PDB. Kumar and Bhadauria (2017) investigated the effect of different media on the growth and sporulation of *Microsporum gypseum.* Various broth media were selected to study the effect on growth rate and sporulation. It was found that Malt Extract Broth showed highest growth of *M. gypseum,* which was 0.407g, followed by Mannitol Salt Broth, Czapek Dox Broth, Richard's Synthetic Broth and modified Sabouraud Dextrose Broth in that order.

Fig. 1 (a-d) represents comparative growth heat maps of *C. versicolor* MTCC 138, *A. oryzae, P. ostreatus* MTCC 142 and *P. chrysosporium* MTCC 787, on different growth media. Heat map is the graphical presentation of data; the change between the colors represents the change in value from low to high. From the preference point of view, it was found that PDB was the most preferred media for *C. versicolor* MTCC 138, where it attained a maximum dry weight of 2.52g (Fig. 4.5 A), whereas YGE supported maximum growth of *A. oryzae.* It is clear from fig 4.5 (C, D) that in case of *P. ostreatus* MTCC 142 and *P. chrysosporium* MTCC 787, highest dry weight was attained in case of PDB.

Table 1: Growth characteristics of standard microbial culture

Culture	Characteristic feature	Media used	Incubation temperature	Incubation period (No. of days)
Coriolus versicolor MTCC 138	Ligninolytic	Glucose yeast extract (GYE)	27°C±2	8-10
Phanerochaete chrysosporium MTCC 787	Ligninolytic	Malt extract agar (MEA)	25°C±2	7-8
Pleurotus ostreatus MTCC 142	Ligninolytic	Glucose yeast agar (GYE)	30°C±2	8-10
Aspergillus niger	Ligno-cellulolytic	Glucose yeast agar (GYE)	30°C±2	7-8

Table 2: Colony diameter and growth rate of fungal cultures on different media

Culture	Medium	Incubation time (days)					
		2		4		6	
		Diameter (cm)	Growth rate (cm day^{-1})	Diameter (cm)	Growth rate (cm day^{-1})	Diameter (cm)	Growth rate (cm day^{-1})
P. ostreatus MTCC 142	PDA	1.0±0.12	0.5	3.8±0.35	0.95	6.8±0.23	1.13
	MEA	2.2±0.30	1.1	4.6±0.23	1.15	7.4±0.11	1.23
	YGEA	1.4±0.23	0.7	3.4±0.53	0.85	6.2±0.42	1.03
	CD	0.8		NS		NS	
C. versicolor MTCC 138	PDA	1.8±0.23	0.9	4.8±0.12	1.20	7.6±0.20	1.26
	MEA	1.0±0.70	0.5	3.0±1.2	0.75	6.0±0.28	1.00
	YGEA	0.6±0.23	0.3	2.8±0.31	0.70	4.6±0.23	0.77
	CD	0.8		0.9		0.86	
P. chrysosporium MTCC 787	PDA	1.0±0.23	0.5	4.6±0.42	1.15	7.2±0.12	1.20
	MEA	1.2±0.31	0.6	2.8±0.31	0.70	4.6±0.34	0.76
	YGEA	1.4±0.23	0.7	4.0±0.58	1.00	6.4±0.35	1.06
	CD	NS		NS		1	
Aspergillus oryzae	PDA	2.4±0.26	1.2	5.2±0.36	1.30	7.8±0.12	1.30
	MEA	2.0±0.34	1.0	4.0±0.31	2.00	7.0±0.12	1.17
	YGEA	1.6±0.50	0.3	3.6±0.23	0.90	5.8±0.42	0.96
	CD	NS		1.03		0.9	

Table 3: Growth profile of fungal cultures on Potato dextrose broth

Incubation Days	Biomass dry weight (g100 ml^{-1})			
	***P. ostreatus* MTCC 142**	***C. versicolor* MTCC 138**	***P. chrysosporim* MTCC 787**	***Aspergillus oryzae***
3	0.21±0.003	0.23±0.003	0.18±0.015	0.33±0.01
6	0.26±0.003	0.35±0.002	0.2±0.018	0.4±0.010
8	0.54±0.045	0.42±0.001	0.46±0.018	0.45±0.010
10	0.66±0.009	0.95±0.002	0.48±0.018	0.57±0.005
12	1.25±0.002	1.01±0.002	0.54±0.017	0.76±0.012
14	1.32±0.025	1.16±0.002	0.85±0.012	0.96±0.001
16	1.59±0.036	1.62±0.002	0.92±0.030	1.02±0.010
18	1.64±0.007	1.92±0.002	1.15±0.030	1.27±0.005
20	1.65±0.018	2.52±0.040	1.09±0.040	1.35±0.010
CD	0.82	0.7	0.84	0.32

Table 4: Growth profile of fungal cultures on Yeast glucose extract broth

Incubation Days	Biomass dry weight (g100ml^{-1})			
	***P. ostreatus* MTCC 142**	***C. versicolor* MTCC 138**	***P. chrysosporim* MTCC 787**	***Aspergillus oryzae***
3	0.16±0.002	0.11±0.001	0.12±0.006	0.35±0.020
6	0.33±0.002	0.29±0.001	0.18±0.012	0.43±0.010
8	0.36±0.005	0.41±0.305	0.26±0.012	0.57±0.01
10	0.53±0.005	0.44±0.002	0.30±0.006	0.68±0.006
12	0.63±0.274	0.51±0.001	0.43±0.017	0.86±0.02
14	0.72±0.018	0.64±0.002	0.45±0.023	1.40±0.020
16	0.99±0.058	0.77±0.002	0.66±0.006	1.50±0.021

18	1.12±0.131	0.85±0.002	0.71±0.017	1.58±0.005
20	1.17±0.202	0.94±0.002	0.78±0.012	1.6±0.006
CD	0.42	0.21	0.4	0.36

Table 5: Growth profiles of fungal cultures on Malt extract broth

Incubation Days	Biomass dry weight (g100 ml^{-1})			
	***P. ostreatus* MTCC 142**	***C. versicolor* MTCC 138**	**P. chrysosporim MTCC 787**	***Aspergillus oryzae***
3	0.18±0.009	0.21±0.012	0.21±0.012	0.59±0.010
6	0.19±0.023	0.32±0.020	0.34±0.014	0.66±0.010
8	0.35±0.032	0.56±0.006	0.38±0.014	0.69±0.005
10	0.41±0.012	0.67±0.006	0.44±0.018	0.77±0.010
12	0.47±0.012	0.79±0.012	0.65±0.023	1.27±0.090
14	0.67±0.024	1.25±0.006	0.7±0.010	1.26±0.012
16	0.92±0.017	1.43±0.012	0.73±0.023	1.47±0.012
18	1.03±0.034	1.56±0.015	0.78±0.020	1.47±0.005
20	1.23±0.009	1.98±0.017	0.98±0.023	1.49±0.017
CD	0.63	0.7	NS	NS

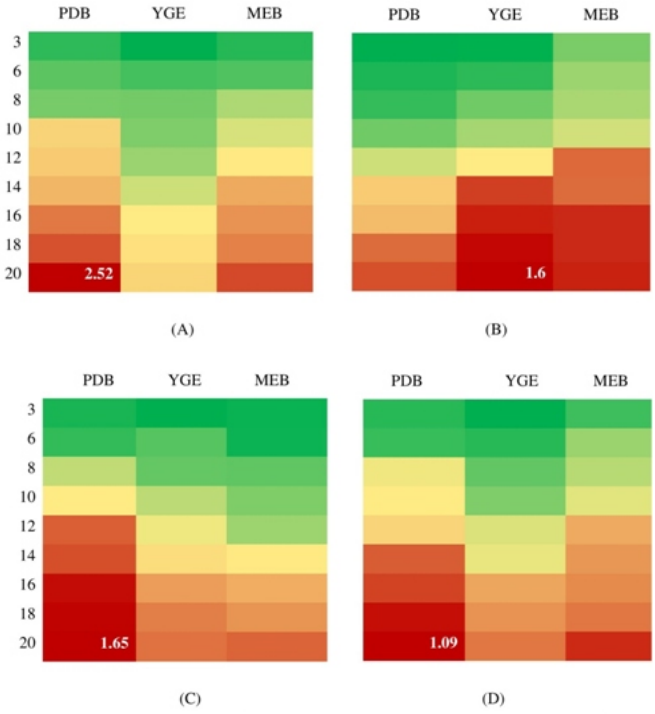

Fig. 1: Heat map of cultures showing comparative dry weight on different media (A) *C. versicolor* MTCC 138 (B) *A. oryzae* (C) *P. ostreatus* MTCC 142 and (D)N*P. chrysosporium* MTCC 787

Conclusion

From the above data, it can be concluded that the nature of a particular medium plays an important role in the growth of fungi. In conclusion, evaluating the suitability of these growth media, potato dextrose agar and potato dextrose broth was the best medium for the growth of the fungi evaluated. As a recommendation, potato dextrose should be used as a medium for fungal growth considering these evidences shown above and it might be necessary to screen more fungi using potato dextrose.

References

Basu S, Bose C, Ojha N, Das N, Das J, Pal M and Khurana S. 2015. Evolution of bacterial and fungal growth media. *Bioinformation* 11(4):182-184.

Collins MM, Nair SB, Vanik DH, Close D, Guy RL, David GI and Peter JW. (2005) Effect of using multiple culture media for the diagnosis of non invasive fugal sinusitis. *American Journal of* Rhinology and Allergy 19(1):41-45.

Devi SK, Kumar MD, Saha J, Devi S and Sinha B. 2018. Screening of Suitable Culture Media for Growth, Cultural and Morphological Characters of Pycnidia Forming Fungi. *International Journal of Current Microbiololgy and Applied Science* 7(8):4207-4214.

Fletcher I, Freer A, Ahmed A and Fitzgerald P. 2019. Effect of temperature and growth media on mycelium growth of *Pleurotus ostreatus* and *Ganoderma lucidum* strains. *Cohesive Journal of Microbiology and Infectious Disease(5):1-9.*

Jo W, Kang MJ,Yoo Y and Seok SJHY. 2010. Culture conditions for mycelial growth of *Coriolus versicolor. Mycobiology 38(3):195–202.*

Kumaran G and Jeya M. 2014. Clinico–Mycological profile of dermatophytic infections. *International Journal of Pharma and Biosciences* 5:1-5.

Mahadevan K and Shanmugasundaram K. 2018. Comparative effect of different culture media on mycelial growth performance of *Pleurotus sapidus. Journal of Pharmacognosy and Phytochemistry* 7(4):874-878.

Nwogu ICG and Elenwo EN. 2012. Comparative evaluation of growth media for the cultivation of fungal cultures. Journal of Plant Pathology and Microbiology 3(6):1-4.

Rawte H and Diwan R. 2011. Growth Response of *Pleurotus* spp. on Different Basal Media and Different pH Levels. *Journal of Ecobiotechnol*ogy 3(4):10-12.

Singh S and Singh G. 2018. Effect of different pH, temperature and media on radial growth of oyster mushrooms *(Pleurotus djamor*) *Bulletin of Environment, Pharmacology and Life Sciences* 7:73-77.