Data Analysis of Fungal Mycelial Radial Growth on Different Nutritional Media Using Statistical Tools.

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ABSTRACT

The effect of different nutritional media on the growth of two fungi of Ganoderma species was studied. The two fungi sp. are referred to as MTCC GANO & MG-GANO. Mycelial growth was studied on 15 different agar media on 100 mm petriplates. The mycelial growth rate in terms of diameter of mycelial growth in mm/day was measured after inoculation of 6mm circular fungal plug at the center of the petriplates. The measurements for each media were done in triplicates after every 24 hours. Days taken for the full growth (days taken for the mycelial growth to fill the entire petriplates) was noted. Statistical analysis was carried out on this data using ANOVA technique. From this analysis it was concluded that there is a significant difference in the growth amongst different nutritional media.

Keywords: Mycelial growth, ANOVA technique, Nutritional media, Fungi

INTRODUCTION

It is a well known fact that different fungi require different growth conditions (M. L. Lomberh, 2002).There is a wealth of information on growth requirements for the type species *Ganoderma lucidum*. Use of various nutritional media, growth conditions & their effects on the growth of *Ganoderma lucidum* & some of the other species of *Ganoderma* has been published in literature. (Z Nasreen, 2005, Saleh Ahmed, 2009, CHIU-YEH WU, 2008). However there is no evidence that show even the same genus requires the same growth & nutritional factors. In addition isolates of same species from different regions may not grow under the same conditions. In fact studies show that both fungi of same species & even the same genus require different growth & nutritional factors (Ofodile etal, 2005). More so, there is a significant variation in mycelium growth rate even in different strains of the same fungi grown in a media. (**A**. J. Kakon, 2009.)

Hence to know the optimum condition for any new isolate it becomes imperative that growth study under diverse conditions be done & not depend on the data of even similar fungi available in the literature.

The effect of different nutritional media on the growth of two fungi of *Ganoderma* species was studied. The two fungi species is referred to as MTCC GANO & MG-GANO. Fifteen different agar media were taken in 100 mm petriplates & the mycelial growth rate in terms of diameter of mycelial growth in mm/day was measured after inoculation of 6mm circular fungal plug at the center of the petriplates. The radial measurements for each media were done in triplicates after

every 24 hours. Days taken for the full growth (days taken for the mycelial growth to fill the entire petriplates) was noted. Statistical analysis was carried out on this data using ANOVA technique.

The quality & quantity of mycelial production can change with the change in the media composition (Fang QH, Zhong JJ 2002, Leandro Papinutti,2010). The media that may support growth may or may not necessarily enhance the production of components of our interest. In addition, increase of certain metabolites in *Ganoderma sinense* has been reported under certain growth conditions (Gao-Qiang, *2010.*). Chiu-Yeh Wu, 2008 S.W. Kim, 2002 demonstrate the effect of different media have varying effect on the composition of the fungi. All these studies indicate that varying the growth conditions, affects the metabolism there by, producing certain metabolites in varying concentrations. So it is possible that the mycelia may show biological activity when grown on one type of growth media while it may not be active when grown on other. Also cultures varying in the age have shown difference in their metabolite concentration. (Leandro Papinutti, 2010)

Using statistical tools (using SPSS Software) the media were compared with respect to their effect on the mycelial growth.

MATERIALS AND METHODS

Study of mycelial growth on different nutritional media:

Total fifteen different agar media were investigated for their effect on mycelial growth. After preparation all the media were autoclaved at 15 pounds for 15 minutes. As the optimum pH obtained for MG-GANO isolate and MTCC GANO was 5.5, the pH of all the media for MGGANO and MTCC GANO was taken 5.5. The optimum temperature range for both the cultures was between 25-35 ºC, so the incubation of both cultures was done at 30ºC temperature.

A 6 mm diameter agar growth plug was removed from 5 day-old cultures grown on potato dextrose agar (PDA) and placed in the centre of a new plate containing 20 ml of different media. This was done in triplicates.

For all inoculations, special care was taken of growth plug that was removed from the mother culture for placing in the media plate. It was removed from the same radial distance from the colony center, in order to guarantee that different inoculate contain the same amount of mycelium at the same stage of development.

The Radial growth of mycelia on each Petri plate was measured every 24 hours after inoculation in millimeter. Measurements were done in 3 directions and the average value was calculated from these measurements. (Ahmed Imtiaj, 2008, Miyashira, C.H, 2010). The measurements were carried out till the mycelial growth reached the perimeter of the plate i.e. the Petri plates was full.

Media for growth study:

Two basal media were used for this study. The composition of the first basal media, designated as **BM-1**is given in Table 1. This was the basal medium for the carbon sources testing.

PDA was taken as the second basal media designated as **BM-2.** This media was prepared according to manufacturers (Himedia) specifications, where in 39 grams of PDA power was dissolved in 1 liters of distilled water.

Both the basal media were modified as shown in the Table 2 & were investigated for their effect on the mycelial growth & their ability to produce antimicrobial activity.

In all 15 different media (Table 2) were investigated. After preparation all the media were autoclaved at 15 pounds for 15 minutes.

Table-1 Basal media no. 1 composition & was designated as BM-1

Carbohydrate source*	**50.0g**
Malt extract	**10.0g**
Yeast extract	**2.0g**
Bacto-peptone	**2.0g**
KH2PO4	**5.0g**
MgSO4	**2.5g**
Agar-agar	**30g**
Water	**1000ml**
pH	**5.0**

Table-2 media used for growth study:

Sr.No of media	MEDIA		pH
1	BM-1+ GLUCOSE		5.5
8	BM-1+FRUCTOSE		5.5
3	BM-1+MALTOSE		5.5
2	BM-1+LACTOSE		5.5
9	BM-1+STARCH		5.5
10	BM-1+GLUCOSE+ WHEAT STRAW (0.5g %)	finely ground wheat straw	5.5
11	BM-1*+ WHEAT STRAW (0.5g %)	finely ground wheat straw	5.5
12	BM-1+GLUCOSE +COTTON STRAW (0.5g %)	finely ground cotton straw	5.5
13	BM-1*+ COTTON STRAW (0.5g %)	finely ground cotton straw	5.5
14	BM-1+GLUCOSE + UREA (0.5g %)		5.5
15	BM-1+GLUCOSE + (NH4)2SO4 (0.5g %)		5.5
4	PDA(BM-2)		5.5
5	PDA(BM-2)+ MALT EXTRACT (0.5g %)		5.5
6	PDA(BM-2)+ YEAST EXTRACT (0.5g %)		5.5
7	PDA(BM-2)+ PEPTONE EXTRACT (0.5g %)		5.5

In case of media number 11*& 13* no carbohydrate source was added.

Statistical analysis:

The results thus obtained for MGSC-GANO and MTCC-GANO was analyzed using statistical tools.

The number of days taken for the mycelial grow to fill the plate was noted. Using ANOVA technique (using SPSS software) it was attempted to find which media gives significantly faster growth.

Using statistical tools the media were compared with respect to their effect on the mycelial growth and their ability to produce antimicrobial activity against *S.aureus and B.subtilis.*

RESULT AND DISCUSSION

The Table-3 and Table 4 show ANOVA table for MGSC-GANO and MTCC-GANO respectively, which shows significant F-ratio hence we may conclude that at least one medium, gives significantly faster growth (or slower growth).

Table-3 Anova

Dayfull for MGSC-gano.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	131.644	14	9.403	204.518	.000
Within Groups	1.333	29	.046		
Total	132.977	43			

Table-4 Anova

Day full for MTCC-gano

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	242.027	14	17.288	126.275	.000
Within Groups	3.833	28	.137		
Total	245.860	42			

To decide for the better media Tuckey-cramer post-hoc analysis is used, the results are presented in table 5 and table 6. By this method all 15 base media at MGSC-GANO is divided in to 6 homogenous sub groups. The first sub group indicate that media 9 (BM-1+STARCH) and media 11 (BM-1*+ WHEAT STRAW (0.5g %)) gives significantly fastest growth (plates were full on

6th day) as compared to all other media. Whereas media 14 (BM-1+GLUCOSE + UREA (0.5g%)) gives slowest growth (plates were full on 13th day).

Table-5 Homogenous subgroups with respect to day full growth on different media for Mgsc-gano**.** TukeyHSDa,b

MEDIA		**Subset for alpha = 0.05**					
	N	1	2	3	4	5	6
9.00	3	6.0000					
11.00	3	6.0000					
3.00	3		7.0000				
4.00	3		7.0000				
5.00	3		7.0000				
6.00	3		7.0000				
13.00	3		7.0000				
7.00	3			8.0000			
8.00	3			8.0000			
10.00	3			8.0000			
12.00	3			8.0000			
1.00	3				8.6667		
15.00	3					9.6667	
2.00	2					10.0000	
14.00	3						13.0000
Sig.		1.000	1.000	1.000	1.000	.848	1.000

By Tuckey-cramer post-hoc analysis the MTCC- Gano(Table 6) is divided in to 5 homogenous subgroups as shown in the table 6. Media 4 to 9, 11 and 13 gives full growth in 6 days which is significantly least (i.e. fastest growth) as compared to all other media. Whereas media 14 gives significantly slowest growth, it took almost 14 days to full the plate with the growth of Ganoderma.

Table-6 Homogenous subgroups with respect to day full growth on different media for Mtcc-gano.

TukeyHSDa,b

Media		Subset for alpha = 0.05				
	N	1	2	3	4	5
4.00	3	5.0000				
5.00	3	5.0000				
6.00	3	5.0000				
9.00	3	5.0000				
7.00	3	6.0000				
8.00	3	6.0000				
11.00	3	6.0000				
13.00	3	6.0000				
3.00	3		7.6667			
10.00	3		8.0000	8.0000		
12.00	3		8.0000	8.0000		
2.00	2		8.5000	8.5000	8.5000	
1.00	2			9.0000	9.0000	
15.00	3				9.3333	
14.00	3					14.0000
Sig.		.145	.370	.145	.370	1.000

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