

# VALIDITY OF MYCELIAL GROWTH ON MALT EXTRACT AGAR AND COMPOST AS SELECTION CRITERIA FOR INITIAL SCREENING OF GENOTYPES FOR YIELD AND QUALITY IN *AGARICUS BISPORUS*

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## ABSTRACT

Sixty-three fertile single spore isolates (SSIs) representing five strains were screened for radial growth on Malt Extract Agar (MEA) medium, downward linear growth on compost and yield. The morphological traits like size of gill, pileus and stipe were also recorded in the above isolates. There was no significant correlation of yield with radial growth on MEA ( $r = -0.124$ ) suggesting that the growth on MEA in petriplates cannot be an indicator of yield performance of the genotype. In another study on seventeen isolates conducted earlier, no significant association of yield was recorded with radial growth on malt, Coon's and yeast potato dextrose agar. The downward linear growth on compost, however, showed highly significant relationship with yield ( $r = 0.771$ ;  $y = 0.33x - 10.01$ ;  $R^2 = 0.59$ ) suggesting that mycelial growth on compost can be used to predict the yield potential and at least can be used to reject the isolates. On the other hand, radial growth on MEA showed significantly negative interrelationship with gill ( $r = -0.381$ ) and stipe size ( $r = -0.310$ ).

**Keywords:** *Agaricus bisporus*, single spore isolates, inter-relationship, path analysis

## INTRODUCTION

Conventional breeding in *Agaricus bisporus* is time consuming due to secondary homothallic nature of the species, absence of clamp connections in mono and dikaryons, and lack of well-defined morphological differences in fertile and non-fertile cultures. One of the simplest techniques for breeding of the strain is to isolate and evaluate single spore isolates. This gains importance while screening SSIs obtained from a hybrid. The frequency of high yielding isolates is low and it is practically impossible to screen all the single spore isolates for their yield performance. It is thus important to look for selection criterion at early stages. Non-significant differences between mycelial growth on media and yield have been reported earlier [1, 2] implying that growth on agar media cannot be an indicator of yield potential. In the present study 63 isolates have been evaluated for their growth on malt extract agar, downward linear growth on sterilized compost and yield, and their inter-relationships have been studied to identify selection criterion for yield and quality of mushrooms.

## MATERIAL AND METHODS

Spore print of five strains viz. A-4, A-6, A-94, S-11 and U-3 were taken and in total 129 single spores were isolated. Sixty-three of these, used for the present study, were cultured on Malt Extract Agar (MEA) and their spawn was prepared on wheat grains following standard practices. 20g pasteurized compost was filled in 25 (dia) x 180 mm sized test tubes and sterilized at 22 p.s.i for 1h. About five

gram spawn was overlaid in each tube and incubated in a BOD incubator at 25±1°C in vertical position for study of downward linear growth. Growth was measured on four sides after 20 days of each tube and averaged. Each isolate was replicated thrice for statistical analysis. The radial growth on malt extract agar medium was measured along both horizontal and vertical axis in mm. The yield was evaluated in bags (10 kg pasteurized compost per bag prepared using wheat straw and chicken manure). Three replication of each isolate were maintained. Yield data was taken up to four weeks of cropping. The morphological data i.e. stipe length, pileus diameter, gill size, firmness were recorded on 10 randomly selected mushrooms from each isolate. The correlation coefficients and path analysis of radial growth on MEA, downward linear growth, morphological traits and yield was done using standard methods.

## RESULTS AND DISCUSSION

The average, standard deviation and coefficient of variation in 63 isolates for different traits viz., radial growth on malt extract agar, downward linear growth, morphological traits (figure 1) and yield are presented in table 1. The highest variation was observed in radial growth on malt extract agar whereas least variation was observed for pileus size.

**Table 1:** Mean, standard deviation and coefficient of variation of different traits of 63 single spore isolates from five strains

Mean	SD	CV
Downward linear growth (mm)	68.899	10.293
Linear growth on MEA (mm)	16.67	7.43
Gill size (mm)	20.44	3.50
Pileus size (mm)	35.40	1.46
Stipe size (mm)	20.23	3.17
Yield (kg/100 kg compost)	12.807	4.421



**Figure 1.** Variation in morphological traits of single spore isolates

The correlation coefficients among traits viz. yield, downward linear growth, gill size and stipe size were found to be positive and significant/highly significant in all combinations. The radial growth on malt extract agar had significant negative association with gill size and stipe size and negative, though non-significant, with yield.

**Table 2:** Correlation matrix of different traits of 63 single spore isolates from five strains

	<b>Downward linear growth</b>	<b>Linear growth on MEA</b>	<b>Gill Size</b>	<b>Pileus Size</b>	<b>Stipe Size</b>	<b>Yield</b>
Downward linear growth	1.000	-0.097	0.301*	-0.082	0.424**	0.771**
Linear growth on MEA		1.000	-0.381**	-0.010	-0.310*	-0.124
Gill size			1.000	-0.161	0.672**	0.467**
Pileus size				1.000	-0.212	-0.073
Stipe size					1.000	0.618**
Yield						1.000

\* p< 0.05, \*\* p<0.01

On partitioning these correlation coefficients using path analysis (Table.3), it can be seen that downward linear growth has a dominant direct effect on yield along with small indirect effect via stipe length. The direct effect of stipe length is only 0.329 and the high correlation coefficient of 0.618 included indirect effect of 0.26 via downward linear growth. The radial growth on MEA has almost zero direct effect on yield and the little non-significant correlation value of -0.124 was due to indirect effect via stipe size.

**Table 3:** Direct and indirect effects of correlation of different parameters with yield

	<b>Downward linear growth</b>	<b>Linear growth on MEA</b>	<b>Gill Size</b>	<b>Pileus Size</b>	<b>Stipe Size</b>	<b>Corr Coef with yield</b>
Downward linear growth	<b>0.616</b>	-0.008	0.030	-0.005	0.138	0.771
Linear growth on MEA	-0.060	<b>0.078</b>	-0.039	-0.001	-0.102	-0.124
Gill Size	0.184	-0.030	<b>0.102</b>	-0.010	0.221	0.467
Pileus Size	-0.050	-0.001	-0.015	<b>0.062</b>	-0.069	0.467
Stipe Size	0.260	-0.025	0.067	-0.013	<b>0.329</b>	0.618

From the correlation coefficients apparently there seems to be highly significant negative association between stipe size and growth on malt extract agar. However, on partitioning, it can be seen that direct effect of radial growth on malt extract agar on stipe size is only -0.104 against the correlation value -0.310 (Table.4).

**Table 4:** Path analysis for stipe vs other traits

	<b>Downward linear growth</b>	<b>Linear growth on MEA</b>	<b>Gill Size</b>	<b>Pileus Size</b>	<b>Yield</b>	<b>Correlation with stipe</b>
Downward linear growth	<b>-0.073</b>	0.010	0.126	0.010	0.350	0.424
Linear growth on MEA	0.007	<b>-0.104</b>	-0.160	0.001	-0.055	-0.310
Gill Size	-0.022	0.039	<b>0.421</b>	0.019	0.214	0.671
Pileus Size	0.006	0.001	-0.067	<b>-0.120</b>	-0.032	-0.212
Yield	-0.056	0.012	0.198	0.008	<b>0.455</b>	0.618

In earlier studies involving former author, it has been observed that there is no significant association of radial mycelial growth on different media (like Malt Agar, Coon's Agar, Compost Agar and Yeast Potato Dextrose Agar) and the yield (Table.5). However, downward mycelial growth had significant positive association. These results were based only on small sample of 17 SSIs of a single strain and were thus only indicative. The present study based upon sixty-three isolates from five strains has verified the earlier results as there is no association of yield with radial growth on media and the correlation coefficient between downward linear growth and yield is highly significant and positive.

**Table 5:** Correlation coefficients of mycelial growth of 17 single spore isolates of strain S-11 on different media, their downward growth on autoclaved compost and their yield

	<b>Malt agar</b>	<b>Coon's agar</b>	<b>Compost agar</b>	<b>YPDA</b>	<b>Downward linear growth</b>	<b>Yield</b>
Malt agar	1.000	-0.012	0.368	0.375	-0.152	-0.238
Coon's agar		1.000	0.403	-0.044	0.399	0.110
Compost agar			1.000	0.358	0.207	0.357
YPDA				1.000	-0.317	-0.278
Downward linear growth					1.000	0.713**
Yield						1.000

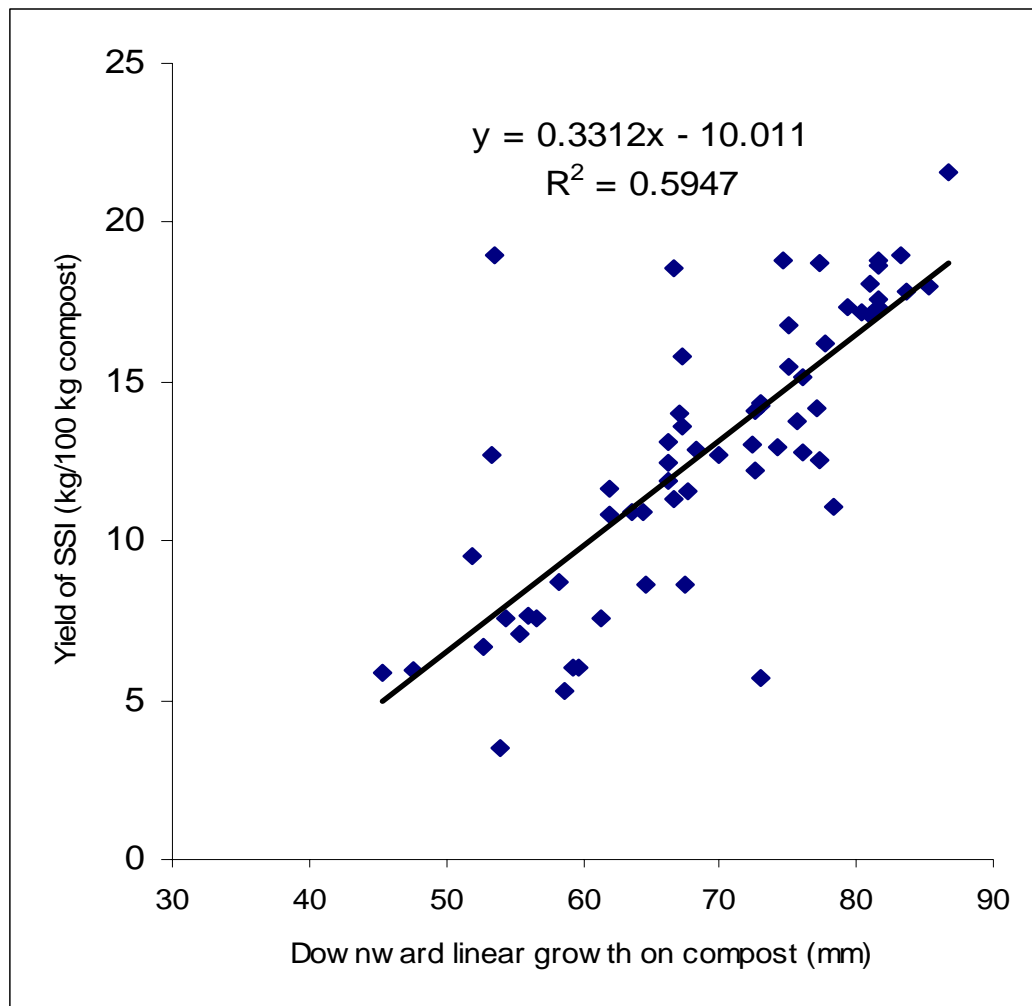
\*\* p<0.01

Analysis of another data of growth rate on media, pileus-stipe-weight-ratio, laccase activity of 21 single spore isolates obtained from a single strain also showed no significant association among these traits (Table-6). Hence, from repeated trials on radial growth on media and yield, it can be inferred that mycelial growth rates on agar are not indicator of yield potential of a culture and thus cannot serve as a selection criteria.

**Table 6:** Correlation coefficients of Growth rate, Pileus/Stipe weight ratio, Laccase activity and Yield

	Growth rate	P/S weight ratio	Laccase activity	Yield
Growth Rate	1.000	-0.055	-0.398	-0.175
P/S weight ratio		1.000	0.234	-0.159
Laccase activity			1.000	0.148
Yield				1.000

On the other hand, the earlier as well as present studies support that downward mycelial growth on sterilized compost can serve as selection criteria while handling a large number of SSIs. Based on the significant association between downward linear growth and yield, the yield can be predicted ( $R^2 = 0.595$ ) with fair accuracy using the regression equation given in figure 2.



**Figure 2.** Regression equation and  $R^2$  value of downward linear growth and yield of 63 SSIs from five strains of button mushroom

## **CONCLUSIONS**

The criteria of downward linear growth can be used to reject 80% or more single spore isolates thereby reducing the total work load for evaluation of SSIs for their yield performance. The growth on malt extract agar though apparently appears to have negative association with stipe length and gill size but partitioning this correlation into direct and indirect effects indicates that the association may not be precisely valid as selection criteria for traits like gill size and stipe length. Even though, association of quality traits like gill size and stipe length with radial growth on MEA is significant, but the degree of association is relatively low and path analysis also shows low direct effect of growth on MEA on stipe size. Hence, detailed studies are required to develop selection criteria for quality traits.

## **REFERENCES**

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