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SUITABILITY OF SCREENED FUNGI FOR SOLID STATE FERMENTATION

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ABSTRACT

Seven fungi were screened to detemine their suitability for solid-state fermentation. In a 5-day submerged fermentation *Aspergillus niger, Aspergillus oryzae, Fusarium oxysporum, Rhizopus isolate, Armillaria mellea* and *Rhizopus sexualis* recorded 166.49, 122.60, 50.58, 23.94. 17.94 and 39.80 mg/ 100 ml biomass production respectively.

***Pleurotus ostreatus* did not show any growth. However the colony radial growth rates of *A. niger, A. oryzae, F. oxysporum* and *P. ostreatus* varied from 10.50-76.88 mm over a 7-day growth period. The best colony radial growth rate of 76.88 mm was obtained for *A. niger* while 49.83, 73.83 and 50.50 mm were obtained for *A. oryzae, P. ostreatus* and *F. oxysporum* respectively. A positive correlation was obtained between colony radial growth rates and rate of starch utilization by the fungi. The overall result indicated that *A. niger* and *A. oryzae* had superiority over other fungi for all the parameters studied. They are therefore recommended for future studies of protein enrichment of starchy substrates by solid state fermentation.**

Key words: Fungi, Biomass, Colony radial growth, Solid-state fermentation

Short title: Screening of fungi

INTRODUCTION

The concept of using micro-organisms in enhancing the nutritive value of plant and animal products is not entirely a new one. Foods and feeds such as cheese, yoghurt, and silages have high content of micro-organisms to which their nutritional properties are due in part (Bellamy, 1976). The biotechnological techniques involved are gradually being introduced in the field of animal nutrition all over the world. Some fungi exhibit amylolytic activities hence their suitability in the protein enrichment of starchy and high fibre containing substrates. Most fungi are capable of degrading a wide variety of substrates (Sachollè and Lösel 1995), and their roles in the nutrition of human beings and animals have been established (Yang, 1988; Padmaja and Balagopalan, 1990; Yang *et al.,* 1993). Colony radial growth rates have been a quick method to determine suitability of *Aspergillus oryzae* and *Rhizopus oligosporus* for solid state fermentaion (Mitchell *et al.,* 1988). The present study was carried out to assess colony growth and clearing rates as a method for determining the suitability of selected fungi for solid state fermentation.

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MATERIALS AND METHODS

Aspergillus niger, Aspergillus oryzae, Fusarium oxysporum, Rhizopus isolate, Armillaria mellea, Rhizopus sexualis and *Pleurotus ostreatus* were used as the test organisms. These organisms were obtained from the culture collection of the Department of Animal and Plant Sciences, University of Sheffield, United Kingdom. The test organisms were cultivated at 25°C on malt extract agar slants containinig (g/l) Lab malt extract agar, 20.0, Lab agar No. 2, 10.0 $NaNO_3$, 2.0, KH_2PO_3, 1.0 $MgS0_4.7H_20.5$. The spores were harvested with a Tween 80 solution (10 ml, 0.01% v/v) which was then adjusted to give 10^7 to 10^8 spores per ml with sterile water. The cultures were sub- cultured every four weeks. However for *F. oxysporum* and *A. mellea* that did not produce spores a sterile cork borer was used to cut a 5mm disc from the margin of the colony culture grown on solid media in a petri dish.

Trial 1

In this trial the biomass production abilities of the selected organisms in submerged fermentation were studied. The fermentation was carried out in 250 ml Erlenmeyer flasks. The liquid medium contained soluble starch, 20.0, $NaNO_3$, 2.0, KH_2PO_4, 1.0, $MgSO_4.7H_2O$, 0.5g/litre. Fifty (50) ml of the medium was dispensed into the flasks and inoculated with 5 ml of the separate inocula accordingly. A control without inoculum but with sterile distilled water was used. Each inoculation was replicated three times. The flasks were incubated at 25°C for five days but each flask was gently swirled every 24 h.

At the end of the fermentation period the contents of the flasks were filtered under pressure with the aid of the Buchner funnel. The filter papers were then re-weighed with the biomass and oven-dried at 60°C for 24 h. The weight of the biomass was determined by difference.

Trial 2

The ability of the fungi, *A. niger, A. oryzae, P. ostreatus* and *F. oxysporum*, to utilize starch was tested through the iodine test. The fungi were maintained on malt extract agar. A disc of agar and mycelium was cut approximately 4 mm from the perimeter of a colony using a sterile cork borer, and then point-inoculated in 9-cm petri dish containing 2.2% starch agar, containing 20g of BDA starch, 10g of Lab M agar No. 2, 2g of $NaNO_3$, 1g of KH_2PO_3 and 0.5g of $MgSO_4.7H_2O$. Each treatment was replicated 21 times, and incubated at 25°C. Three radial growth rates per fungus were measured with a transparent millimeter ruler at right angle. This was done on a daily basis for one week. Two diameters were measured to give the mean diameter for each colony. Where unequalled diameters were observed the longest and the shortest diameters were measured and the average taken to give the mean diameter for that colony. The rate of starch break down (utilization) was measured by adding 4-5 drops of Grams iodine solution on the growing fungus and the diameter of the cleared area measured as previously described for colony radial growth rate.

RESULTS AND DISCUSSION

The biomass production of the selected fungi is shown in Fig. 1. The values obtained varied from 17.94 to 166.49 mg/100 ml over one week period. *A. niger* recorded the highest among all the fungi while *A. mellea* recorded the lowest value. *Pleurotus ostreatus* did not show any growth in the submerged fermentation. This may not be unconnected with the nutitional requirements of the fungus.

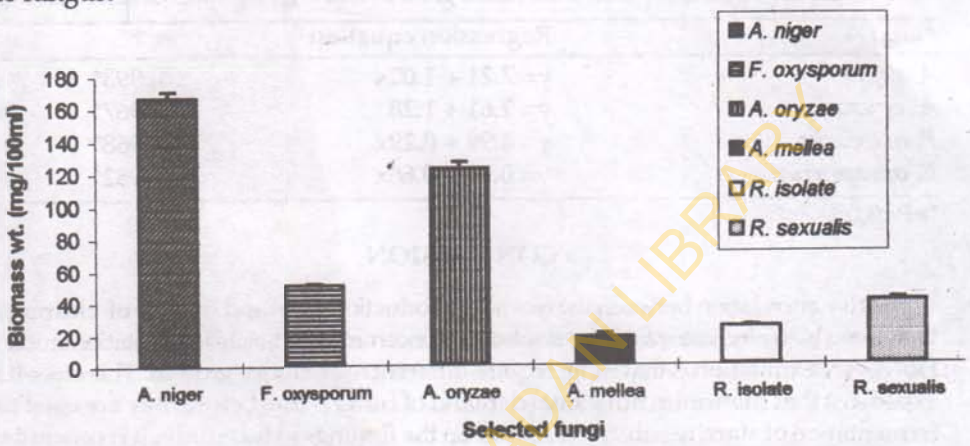

Fig 1: Biomass production of selected fungi grown in a liquid culture

The colony radial growth for the selected fungi varied from 10.50-76.88 mm over a period of one week (Table 1). At the end of the 7th day, the best colony radial growth of 76.88 mm was obtained for *A. niger* while the values of 49.83, 73.83 and 50.50 mm were obtained for *A. oryzae, P. ostreatus* and *F. oxysporum*, respectively. Radial colony growth rate, a function of biomass production, is not an ideal indicator for selecting fungi for solid-state fermentation (Bull and Trinci, 1977). The rate of starch utilization was therefore estimated and this showed a positive correlation with biomass production level.

Table 1. Colony radial growth and clearing rates of selected fungi for suitability of fermentation (mm)*

Days	*A. niger*		*A. oryzae*		*P. ostrearus*		*F. oxysporum*	
	colony diameter (mm)	clearing diameter (mm)	colony diameter (mm)	clearing diameter (mm)	colony diameter (mm)	clearing diameter (mm)	colony diameter (mm)	clearing diameter (mm)
1	17.83	11.17	11.33	14.00	10.50	7.33	15.00	13.67
2	26.17	20.83	29.00	26.00	16.83	7.33	21.33	19.17
3	43.33	35.50	32.50	37.50	22.67	12.83	26.50	23.67
4	55.00	49.67	32.83	46.00	30.67	16.50	30.50	31.50
5	68.33	57.67	40.67	50.67	47.17	18.83	33.17	36.50
6	75.00	69.17	51.33	62.17	63.33	24.50	40.83	39.50
7	76.88	75.67	49.83	62.67	73.83	24.67	50.50	47.67

* The values obtained are means of triplicates determinations.

The result of the biomass production trial showed that *A. niger* had the highest biomass production followed by *A.oryzae A. mellea* had the least rhizomorph biomass production and this can be attributed to absence of alcohol as carbon source (Weinhold and Garraway, 1965). Regression analysis showed that there is a positive correlation between the colonial radial growth and clearing rated of the fungi grown on starch agar (Table 2)

Table 2: Linear regression of colonial radial growth and clearing rates of selected fungi

Fungi	Regression equation	r
A. niger	y= 7.21 + 1.02x	0.993*
A. oryzae	y= 2.61 + 1.28	0.967*
P. ostreatus	y= 4.99 + 0.29x	0.968*
F. oxysporum	y= 0.99 - 0.66x	0.982*

*=P<0.05

CONCLUSION

A positive correlation between the biomass production level and the rate of clearing is therefore a better indicator for fungus selection concerning solid-state fermentation studies. However, a limitation is that fungi require different nutrients for growth. Therefore it is expedient that minimum nuritional demand of fungi is met before they are used for fermentation of starchy substrates. Based on the findings in this study, it is concluded that *A. niger* and *A. oryzae* may be better choices of fungi for future solid state fermentation of starchy substrates.

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