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Comparison of cassava, yam and potato dextrose agars as fungal culture media

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Abstract

In experiments in vitro at 20 and 30°C the fungi *Phytophthora palmivora, Aspergillus melleus, Thielaviopsis paradoxa, Pestalotiopsis versicolor* and *Curvularia pallescens* showed a better mycelial growth on cassava dextrose agar than on potato dextrose agar and yam dextrose agar. This also applied to sporulation, except for the last two fungi at 20°C, which sporulated best on potato dextrose agar.

Introduction

Potato tubers are often used to prepare the medium potato dextrose agar for culturing a wide range of fungi. On this medium, however, *Pythium debaryanum* Hesse does not grow readily and sporulation of *Phytophthora palmivora* (Butl.) Butl. was found to be very poor (Weststeijn, unpublished).

In tropical countries other starch containing tubers, like cassava (*Manihot utilissima*) and white yam (*Dioscorea rotundata*), can possibly replace the potato in a general purpose culture medium. Hislop and Park (1962), for instance, prepared a very good medium from cassava tubers to culture *Ph. palmivora.* Maduewesi and Nwauzo (1966) obtained good mycelial growth and sporulation of several fungi on a medium prepared from *Dioscorea dumentorum.*

The experiments to be reported here, were performed to test the suitability of cassava and yam tubers as ingredients for general purpose culture media in tropical countries.

Materials and methods

Preparation of culture media

1. Cassava dextrose agar (CaDA). Cassava tubers, *Manihot utilissima*, variety 53101, selected by the Nigerian Federal Department of Agricultural Research, were peeled, cut into chips, dried overnight at approximately 55 °C, ground and passed through a 30 mesh sieve. The resulting fine powder was preserved and used as stock.

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A quantity of 135 g of this powder was soaked in approximately 500 ml deionized water at 60 °C for 15 min. The suspension was then filtered using a fine cloth. (At a temperature of 62 °C and above the starch grains would swell, so that subsequent filtration of the broth would be impossible). To the filtrate 20 g of dextrose and 12 g of plain agar (Oxoid No. 3) were added, the volume made up to 1000 ml and boiled for proper dissolution of the agar. The medium was then pipetted into test tubes in aliquots of 10 ml per test tube and autoclaved at approximately 1 atm. for 20 min.

After autoclaving, each test tube was emptied into a pair of Petri dishes, taking care to distribute equally through the medium any precipitate which had been formed.

2. Yam dextrose agar (YDA) was prepared similarly using tubers of *D. rotundata*, variety 3039 of the Federal Nigerian Department of Agricultural Research at Ibadan. For complete sterilisation this medium had to be heated to 100 °C on two consecutive days and autoclaved like CaDA on the third day.

3. Potato dextrose agar (PDA) was made up from Oxoid's dehydrated medium CM 139, using the manufacturer's recipe, and also autoclaved at approximately 1 atm. for 20 min.

The fungi

The vegetative growth and sporulation of the following fungi were tested: *Phytophthora palmivora* (Butl.) Butl., isolated from cacao pods in Western Nigeria; *Aspergillus melleus* Yukawa, isolated from cacao roots in Western Nigeria; *Pestalotiopsis versicolor* (Speg.) Stey., isolated from coffee leaves in Northern Nigeria; *Thielaviopsis paradoxa* (de Seynes) Höhnel, imperfect state of *Ceratocystis paradoxa* (Dade) Moreau, isolated from central leaves of young coconut in Eastern Nigeria and *Curvularia pallescens* Boedijn, isolated from maize leaves in Western Nigeria.

Inoculation and incubation

Each plate was inoculated in the centre with a 9 mm circular disc, taken from the margin of a 7–10-day-old culture of the fungi on PDA (except *Phytophthora palmivora*, which grew on CaDA). One set of cultures was incubated at 20 ° C and another at 30 °C, to allow for the different temperature requirements of the fungi. No light was provided. Each treatment was replicated 5 times.

Measurement of mycelial growth and sporulation

The growth of the colonies was measured daily along 2 perpendicular axes.

Seven days after inoculation, the spores were washed off into deionized water, diluted to a suitable concentration and counted by means of a haemocytometer. A wetting agent (Teepol) was added to the spore suspension of *P. versicolor*. The zoospores of *Ph. palmivora* were liberated from the zoosporangia using the fan method described by Weststeijn (1965) and immobilized by the vapour of 1 % aqueous osmic acid (Hislop and Park, 1962).

As it proved impossible to break the chains of spores of *A. melleus* completely, the conidiophores were counted in a continuous chain of fields of view of the microscope moving along a diameter line from one point at the margin of the colony to the op-

posite. The diameter of each field of view was 1.29 mm. The spores of 2 plates of each treatment were counted. When these duplos diverged too much, more than 1 count of each of the 2 plates were made and the average was taken.

Colour assessment

During the experiments changes in colour of the colonies were assessed visually with the aid of the Methuen Handbook of Colour (Kornerup and Wanscher, 1963).

Results

a. Vegetative growth of the mycelia

The results given in Table 1 show that all 5 fungi grew most rapidly on CaDA, followed by YDA for *Ph. palmivora*. *A. melleus* grew equally well on YDA as on PDA, while the other fungi did better on PDA. In fact *T. paradoxa* grew so well on CaDA that the plates had been overgrown within 3 days, reason why the data given in Table 1 for this fungus represent the diameter increase between the end of the first and that of the second day. The differences indicated above were significant at $P \leqslant 0.05$.

Growth was quickest at 30 °C for 3 of the fungi, i.e. *C. pallescens*, *A. melleus* and *T. paradoxa*. The last fungus even filled the plates at 30 °C within 2 days. At 30 °C growth of *P. versicolor* was slightly retarded and that of *Ph. palmivora* completely inhibited.

Table 1. The mean increase (mm) of the diameter of fungal colonies at 20 and 30 °C after growth on three culture media for 2 and 6 days, respectively.

Fungus	Temperature						LSD (P=0.05)
	20 °C			30 °C			
after 2 days	CaDA	YDA	PDA	CaDA	YDA	PDA	
Curvularia pallescens	19.3	8.6	15.4	37.4	14.1	28.3	
Aspergillus melleus	11.8	9.5	9.1	16.5	16.1	13.8	1.72
Pestalotiopsis versicolor	31.8	24.4	26.2	27.9	18.9	22.2	
Phytophthora palmivora	29.0	21.8	15.9	0	0	0	2.20
Thielaviopsis paradoxa[1]	44.2	19.8	33.6	>45.4	30.2	43.1	3.84
after 6 days							
Curvularia pallescens	49.3	23.6	38.2	66.3	56.5	68.3	
Aspergillus melleus	37.3	31.3	30.5	45.8	40.5	38.8	2.65
Pestalotiopsis versicolor	71.2	64.7	68.7	68.0	47.6	62.9	
Phytophthora palmivora	68.4	57.2	41.6	0	0	0	2.15

[1] Growth for only one day (see text).

Tabel 1. De gemiddelde diametertoename (mm) van schimmelkolonies bij 20 en 30 °C na groei op drie voedingsbodems gedurende 2 resp. 6 dagen.

Table 2. Number of spores in thousands per cm² of colonies of fungi after 7 days growth on three culture media at 20 and 30 °C, respectively.

Fungus	Temperature						Levels of significance between means		
	20 °C			30 °C			none	0.01 ⩽ P ⩽ 0.05	P ⩽ 0.01 remaining combinations
	CaDA (1)	YDA (2)	PDA (3)	CaDA (4)	YDA (5)	PDA (6)			
Curvularia pallescens	8	13	73	801	118	168	(1,2)		
Pestalotiopsis versicolor	18	5	30	225	116	11		(2,6)	
Thielaviopsis paradoxa chlamydospores	65	39	60	151	81	162	(4,6) (1,3) (1,5)	(3,5)	
endoconidia	269	223	529	219	96	175	(4,6) (2,4)	(1,4)	
Phytophthora palmivora (zoospores)	8	0	3	–	–	–			(1,3)
Aspergillus melleus (conidiophores)	0.46	0.23	0.17	0.50	0.36	0.29	LSD (P=0.05) =0.10 LSD (P=0.01) =0.13 LSD (P=0.001)=0.24		

Tabel 2. Aantal sporen per cm² schimmelkolonie na groei gedurende 7 dagen op verschillende voedingsbodems bij 20 resp. 30 °C (× 1000).

b. Sporulation

Based on the spore counts 7 days after the start of the experiments the number of spores, produced by each fungus per cm² of the cultures (for *A. melleus*: the number of conidiophores) was calculated.

The data, presented in Table 2, show that, in general, sporulation was most profuse at 30 °C, except for *Ph. palmivora* and the endoconidia of *T. paradoxa*. At this temperature most fungi sporulated best on CaDA, except for *T. paradoxa*, which did equally well on PDA.

At 20 °C *C. pallescens* sporulated best on PDA, but *Ph. palmivora* and *A. melleus* did best on CaDA. The results of both experiments corresponded so far. For the 2 remaining fungi, however, sporulation at 20 °C on CaDA was best in 1 and on PDA in the other of the 2 replicate experiments, so that the data given in Table 2 for *P. versicolor* and *T. paradoxa* cannot be considered to be conclusive.

As the counts (for assessing the concentration) of the spore densities were made by means of a haemocytometer and thus followed a Poisson distribution, their means had all different variances. Consequently the standard deviations of the difference between each pair of means varied, so that a least significant difference for the whole experiment could not be calculated. This was only possible for *A. melleus*, whose sporulation was assessed by direct counts on the cultures. For the remaining fungi the significance of the difference between each pair of means was calculated separately and is given in the last 3 columns of Table 2.

c. Colour of colonies

The most striking difference in colour between colonies on various media was found with *C. pallescens*, which had a light greyish-green colour on CaDA and PDA, but a reddish one on YDA. For the remaining fungi the media had no clear influence on the colour of the colonies.

There was some obvious influence of temperature, however, on the colour of *A. melleus*, which was light orange at 30 °C irrespective of medium, but whitish yellow at 20 °C; *P. versicolor* was slightly browner in the centre of the colonies at 30 °C than at 20 °C. The colour of the remaining fungi was not influenced by the temperature.

d. Zonation of colonies

P. versicolor formed concentric rings, consisting of concentrations of acervuli, especially at the higher temperature. In *A. melleus* rings were formed by concentrations of sclerotia. Cultures of *C. pallescens* had concentric rings on CaDA and PDA only, whereas those of *T. paradoxa* had them only in the PDA plates. *Ph. palmivora* had formed conspicuous concentric rings on CaDA only, which consisted of closely spaced sporangia.

Discussion

As general purpose culture medium for rapid mycelial growth and sporulation of the test-fungi cassava dextrose agar is to be preferred. PDA only supported mycelial growth equally well after 6 days for *C. pallescens* at 30 °C and *P. versicolor* at 20 °C. The better sporulation on CaDA was most obvious for these 2 fungi at 30 °C and for *A. melleus* at 20 °C. *T. paradoxa* sporulated equally well on PDA, whereas *Ph. palmivora* on the other hand hardly sporulated on PDA.

YDA usually gave less mycelial growth and sporulation than the other 2 media; the slightly higher numbers of sporophores of *A. melleus* on this medium compared to PDA were not significant.

The number of spores per cm^2 is not only influenced by the substratum, but also by the age of the mycelium. As the growth-rate of the fungi was different, the average age of the mycelium, from which spores were sampled, was higher at the moment of sampling for those fungi, which had filled the Petri-dishes before the end of the experiments. This may apply to the chlamydospore formation of *T. paradoxa* on CaDA.

A possible effect of light on zonation cannot be excluded (Rudolph, 1962; Yusef and Allam, 1967; Hendrix, 1967); though the cultures were incubated in the dark, they were taken into the daylight every day when their growth had to be measured.

These experiments have shown that an excellent medium can be prepared from cassava tubers, which is more suitable for many purposes than the widely used potato dextrose agar. The yam tubers proved much less promising.

However, there are some points to be noted, when using cassava dextrose agar. First of all, the chemical composition of the tubers of different cassava varieties was shown to differ widely (Anon., 1966). This may explain why at first many cassava varieties had to be tested at the Cocoa Research Institute of Nigeria (unpublished data), before the variety 53101 was found, which induced moderate mycelial growth

and abundant sporulation in *Ph. palmivora*. Furthermore, this medium gives sometimes rise to sectors of aberrant growth in cultures of *Ph. palmivora* (Weststeijn, 1964), where the mycelium may vary the thickness and sporulation. An important advantage of this medium for tropical countries, however, is the possibility to grow the cassava locally and to reproduce it vegetatively.

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Samenvatting

Vergelijking van cassave-, yam- en aardappelglucose-agar als voedingsbodem voor schimmels.

Bij vergelijking van de invloed van aardappelglucose-agar (PDA), cassaveglucose-agar (CaDA) en yamglucose-agar (YDA) op de mycelium groei en sporulatie van *Curvularia pallescens*, *Phythophthora palmivora*, *Aspergillus melleus*, *Pestalotiopsis versicolor* en *Thielaviopsis paradoxa*, bleek CaDA in het algemeen de beste groei (Tabel 1) en sporulatie (Tabel 2) te geven. Cassaveglucose-agar kan beschouwd worden als een goede algemene voedingsbodem voor het kweken van schimmels. Vooral in de tropische landen kan men hiervan een nuttig gebruik maken. De geschiktste cassavevariëteit moet langs empirische weg geselekteerd worden.

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