An Improved, yam-based, dehydrated agar medium for cultivating fungi

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ABSTRACT

Radial growth of Sclerotium rolfsii on media containing concentrations of 0.4, 0.8, 1.2, 1.6 and 2% yam powder were not significantly different ($P = 0.05$) from each other, irrespective of whether sucrose or glucose was used in the medium. At any of these concentrations, and regardless of the sugar used, radial growth of the fungus was greater than that on Oxoid potato dextrose agar (PDA). Aerial mycelial growth, however, was greater on PDA, and among the yam media, was more profuse on those with 1.6 and 2% yam powder concentrations and least on that with 0.4% concentration of yam powder. Media clarity, however, was best at the lowest yam powder concentration. With either sugar, significantly higher number of mature sclerotia of S. rolfsii formed at 0.8% powder concentration and above. None was formed on media devoid of yam powder. In an additional comparative test with Oxoid Sabouraud glucose agar, 18 phytopathogenic fungi grew well on a medium containing (w/v) 0.4% yam powder, 2% sucrose, 1.5% agar and fortified with 0.2% cowpea powder. This yam-cowpea agar medium is therefore, recommended for supplementary use with imported dehydrated mycological media.

Keywords: Dehydrated mycological media, Sclerotium rolfsii

INTRODUCTION

Even though preparation of mycological culture media from West African food items is well established [6], formulation of such food items into dehydrated, readily utilizable forms was rare until recently when this was reported to be feasible [1]. In that study, powders from a variety of foodstuffs were formulated at 4% (w/v) and this high powder concentration made the media turbid on autoclaving and gelling. Media from white yam (Dioscorea rotundata Poir), cassava (Manihot esculenta Crantz), sweet potato (Ipomea batatas (L.) Lam.) and cocoyam (Xanthosoma saggitifolium (L.) Schott) were, however, clearer but were inferior with respect to fungal growth. The locally formulated media also did not incorporate any other nutrient and it has recently been reasoned that with a few additives, the mycological utility of such media could be enhanced, especially if very low foodstuff powder concentrations are to be used for improved media clarity. Therefore, yam was selected for the present study which had the following objectives.

i. To determine the minimum powder concentration for optimum media clarity and fungal growth
ii. To compare the mycological effect of glucose and sucrose as media additives and
iii. To stimulate aerial fungal growth on the yam media with a cowpea powder additive

MATERIALS AND METHODS

Yam Powder Preparation

Processing of white yam tuber was modified after Awuah [1]. The tuber was peeled, washed and sliced into pieces. A 1000g sample of sliced yam was thoroughly cooked with enough water in a sauce pan and mashed into a semi-solid paste. The paste was transferred into a 35 x 25 x 12 cm aluminium baking pan and oven-dried at 60°C for 48h. During drying, the yam paste was broken up into clumps by hand. The dry product was pulverised with the Waring blendor and subsequently ground with a grinding stone. The powder was passed through a Griffin and George No. 100 sieve and the fine powder stored in a plastic container until needed.

Media Formulation

Quantities of yam powder (0.4, 0.6, 0.8, 1.2, 1.6 and 2.0g) were weighed into 150ml sterilizing bottles. Each was mixed with 2g of sucrose (obtained as granulated table sugar) and 1.5g Oxoid bacteriological agar No. 1. A formulation devoid of yam powder was also included. A parallel set up incorporating glucose was also established. The mixtures were suspended in 100ml tap water, thus establishing media with yam powder concentrations of 0, 0.4, 0.8, 1.2, 1.6, 1.8 and 2.0%. These were autoclaved at 115°C (0.7kg/cm^2 pressure) for 20 min., dispensed

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into 9cm diameter glass Petri plates, stored overnight and tested for their capacities to support fungal growth. Oxoid PDA was included as the control.

Bioassay procedure

Sclerotium rolfsii Sacc., causing wilt of tomato, was isolated and maintained on PDA at 5°C. For the bioassay, fresh, single sclerotial cultures were established on PDA and 7mm diameter mycelial plugs removed from the colony margins, placed, top down, on the media and incubated on a laboratory bench (24-28°C). Four replicate transfers of the fungus were made to each medium. Diameters of the emerging colonies were measured after 24 or 48h. Additionally, the media were visually assessed for clarity. Visual assessment of aerial mycelial growth and sclerotial counts were made after 6 days.

To enhance aerial mycelial growth, an agar medium formulated with 0.4% yam powder, 2% sucrose and supplemented with 0.2% cowpea powder (prepared by stone-grinding dry cowpea seeds and sieving as before) was assayed as above using the following fungi: Sclerotium rolfsii, an Aspergillus sp., Trichoderma viride Pers. ex Fr., Curvularia lunata (Wakk.) Boedjin, Bipolaris maydis (Nisikado) Shoemaker, Botryodiplodia theobromae Pat., Aspergillus niger V. Thiegh. a Pestalotia sp., Nigrospora sphaerica (Sacc.) Mason, Alternaria porri (Ell.), Cif., Fusarium oxysporum Schlect. emend. Snyd. & Hans. f. sp. tracheiphilum, Colletotrichum gloeosporioides Penz., Cercospora henningsii Allesch., Cladosporium fulvum Cke., Pycnosporus sanguineus (Lk. ex Fr.) Murr. Coriolopsis polyzona (Pers.) Ryv., a Trametes sp. and a Mucor sp. Apart from Pycnosporus, coriolopsis and Trametes which were obtained from the Forest Research Institute of Ghana, Kumasi, the rest were isolated from diseased plant parts following standard procedures [11]. Oxoid Sabouraud glucose agar (SGA) was used as the control.

RESULTS

Radial growth per 24h of S. rolfsii on media formulations incorporating sucrose and containing 0, 0.4, 0.8, 1.2, 1.6, 1.8 and 2% yam powder were 2.18, 3.08, 3.12, 3.24, 3.29 and 3.29 cm respectively (Fig. 1). Except for growth on the medium devoid of yam powder, these values were significantly different ($P = 0.05$) from the 2.2cm/24h radial growth obtained on Oxoid PDA. Among the yam media, radial growth did not significantly differ (Fig. 1). A similar trend was observed when glucose was utilized (Fig. 1).

Significantly more mature sclerotia were produced with both sugars on media prepared with 0.8% yam powder and above (Fig. 2A). Sclerotia counts at these concentrations were comparable to those on PDA which had an average of 559 ± 89.4 per plate

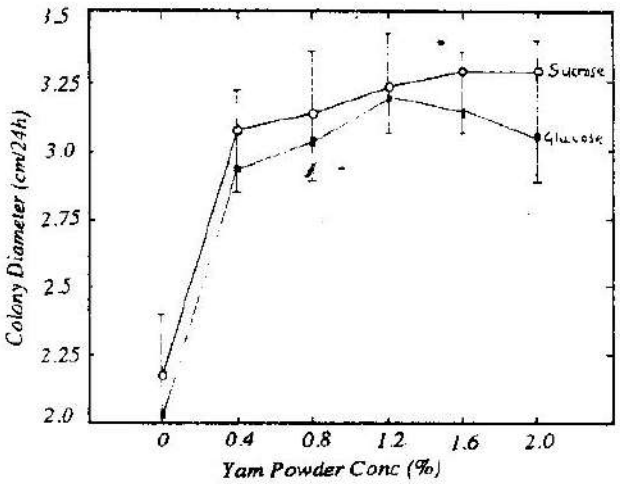

Fig. 1: Radial growth of S. rolfsii on agar media formulations with varying concentrations of yam powder

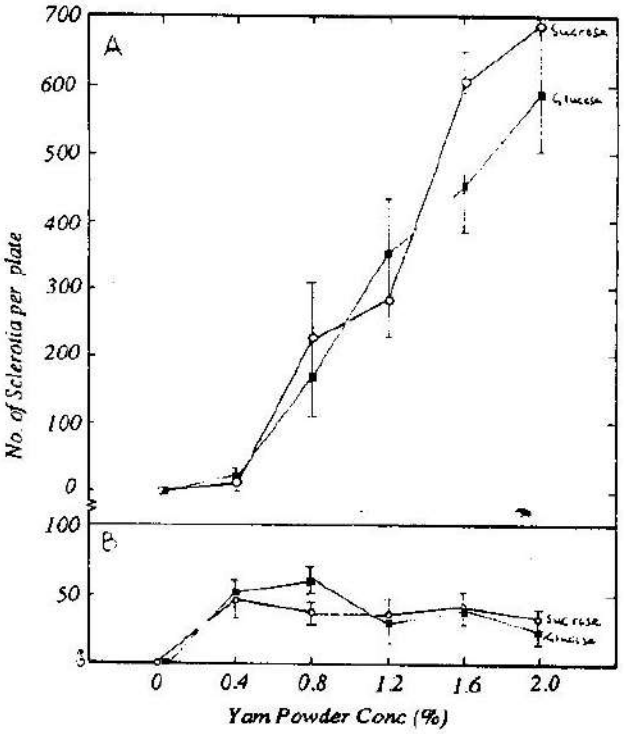

Fig. 2: Sclerotia production by S. rolfsii on agar media formulations with varying concentrations of yam powder after 6 days. A = Mature Sclerotia; B = Immature Sclerotia

after 6 days. In general, immature sclerotia numbers on the yam media did not vary (Fig. 2B).

Aerial mycelial growth of S. rolfsii was greatest on the Oxoid PDA and among the yam media, was more on the media with 1.6 and 2.0% yam powder and scanty on the medium with 0.4% powder. Media clarity, however was impaired at the higher powder

concentrations and enhanced at the 0.4% concentration.

Of eighteen fungi tested on media containing 0.4% yam powder, 2% sucrose, 1.5% Oxoid bacteriological agar and fortified with 0.2% cowpea powder, 6 grew (radially) better than on Oxoid SGA, 6 grew better on the SGA and 6 grew to about the same extent on both media (Table 1). In general, aerial growth of the fungi on the local preparations were enhanced, being generally comparable to that on the Oxoid SGA (Table 1). Six of the fungi could not sporulate on either media.

Table 1
Growth Characteristics of 18 fungi on the Yam - Cowpea medium and Sabouraud glucose agar

Fungi used	Yam - Cowpea medium			Sabouraud glucose agar		
	CD	AG	SP	CD	AG	SP
Sclerotium rolfsii	3.56	+++	+	2.9*	+++	+
Aspergillus sp.	1.19	++ (+)	+	1.58*	+++	+
Trichoderma viride	2.38	++ (+)	+	3.50*	+++	+
Curvularia lunata	1.92	+++	+	1.65*	+++	+
Rhizopus stolonifer	1.81	+++	-	1.52*	+++	-
Botryodiplodia theobromae	3.63	++	-	3.40*	+++	-
Aspergillus niger	1.81	++	+	2.27*	+++	+
Botryodiplodia sp.	0.79	++ (+)	+	1.14*	+++	+
Nigrospora sphaerica	1.93	++ (+)	-	1.24*	+++	-
Corynespora cassiicola	1.82	+++	+	1.80	+++	+
Mucor sp.	2.36	+++	+	2.51*	+++	+
Trametes sp.	1.12	+++	+	1.12	+++	+
Fusarium oxysporum	1.47	+ (+)	+	1.40	+++	+
Alternaria sorii	1.23	+++	-	1.08*	+++	-
Colletotrichum gloeosporioides	1.30	++ (+)	+	1.35	+++	+
Corynespora cassiicola	1.13	++ (+)	-	1.29*	+++	-
Cladosporium fulvum	0.54	+++	+	0.56	+++	+
Pythiomorpha gonapodyides	0.51	+++	-	0.54	+++	-

CD = Colony diameter in cm/24h. Each is an average of four replications.

AG = Aerial growth after 6 days: + = scanty; ++ = moderate; +++ = good

SP = Sporulation after 6 days: + = present; - = absent; for S. rolfsii + indicates sclerotia

Colony diameters in the same row followed by asterisks are significantly different ($P = 0.05$)

DISCUSSION

Previous use of locally prepared dehydrated media containing 4% foodstuff powder [1] was hampered by reduced media clarity, which made it difficult and sometimes impossible, to determine with certainty fungal colony margins from the underside of plates. This problem has been partly overcome in the present study with yam, by using a powder concentration of 0.4%. Even though the medium containing 0.4% yam powder supported radial growth of S. rolfsii, it was unsuitable for aerial mycelial development. This shortcoming was remedied in a number of preliminary experiments in which the yam medium was fortified with 0.2% cowpeas powder. Because more powder (total 0.6%) has been added to the medium, optimum clarity if desired can be obtained if the medium is not overly agitated during plate - pouring. This will leave out much of the yam and cowpea particles which will otherwise reduce media clarity.

The utility of the yam-cowpea medium as a broad spectrum mycological medium has been demonstrated by its capacity to support growth (radial and aerial) of eighteen fungi in a manner generally comparable to Oxoid SGA. Ingold [3] indicated that a good mycological medium must contain all the essential nutrients required for fungal growth. The low nutrient status of yam [7], which appears to limit the mycological usefulness of a medium formulated entirely from the crop, was apparently ameliorated with powder from cowpea, a crop rich in nutrients, especially proteins [9], which is required for mycelial development [8].

Neither glucose nor sucrose had any beneficial effect over each other with respect to fungal growth. Though monosaccharides are excellent sugars for fungal growth, the disaccharide, sucrose, is an equally good source of carbon for several fungi [10]. In most developing countries, sucrose is cheaper than glucose and is more readily obtainable as granulated table sugar. Therefore, its use for media preparation is recommended. If analytical grade of sucrose is obtainable, it would be more preferable.

Inability of some of the test fungi to sporulate was not unexpected since not all fungi form spores readily but may require special light treatments for sporulation (2, 4, 5).

The yam-cowpea dehydrated medium is cheap, and easy to prepare. It is, therefore, recommended for supplementary use with imported mycological media. The cost of a 500g sample of such a medium is estimated at ¢5,500. (£8.5; £1 = ¢650). A similar quantity of an imported broad spectrum mycological medium such as potato-dextrose agar will cost about ¢20,000 (£30.8) if available.

CONCLUSION

A dehydrated mycological agar medium prepared from yam and amended with sucrose and cowpea powder exhibited mycological properties either better than or comparable to those of imported Oxoid Sabouraud glucose agar. This local medium is cheap, easy to formulate and is recommended for supplementary use with imported mycological media.

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