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Short Communication

Growth and cultural characteristics of selected bacteria on Cowpea Agar (*Vigna unguiculata*)

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A general purpose solid bacteriological medium, Cowpea Agar (CPA), was prepared from black-eyed cowpea (*Vigna unguiculata*) for use in classroom non-research practical lessons in microbiology for deprived schools in developing countries. The growth and cultural characteristics of *Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli, Enterobacter faecalis, Stapylococcus epidermidis* and *Salmonella typhi* were studied on this medium and compared with industrially manufactured Nutrient Agar (NA). There were comparable bacterial population growth and colonial characteristics of the test organisms on the CPA and NA. The CPA could be stored for, at least 3 months, in pulverised form. The cost for preparation of one litre of the medium was 58 times cheaper than the industrially-made NA.

Key words: Cowpea, bacteriological medium, colonial characteristics.

INTRODUCTION

Teaching of Microbiology as a subject to students of tertiary institutions in Ghana, and probably in some other developing countries, is strong in theory but rather weak in practical. Student numbers exceed available laboratory spaces during practical periods. Whereas this may be partially solved by student group rotation and time-table adjustments, there is a major constraint with the use of industrially manufactured microbiological media because of their unavailability or prohibitive prices. More so, the use of industrially manufactured culture media for students' practical is of no immediate research value, but for practice only. For this reason, a bacteriological medium that may be made from an affordable local material, storable in a pulverised form for reconstitution, as is done for industrially manufactured media, appears to be welcomed. It is for this reason that a general purpose medium was developed from cowpea (*Vigna unguiculata* (L) Walp (Purseglove, 1995).

Providing 33% of the world's production of proteins (Bliss, 1998), cowpea is an annual crop with indeterminate growth, showing a tendency to be perennial (Haizel, 1972) and with pods containing 6-15 globular or kidney-shaped seeds. The plant contains the soluble anthocyanin and melanin-like pigments responsible for colour in the cowpea (Mann, 1994). Cowpea contains 24.8% protein, 62% soluble carbohydrates, 1.4% fat, 2% mineral salts and 0.2% vitamins (FAO, 1982; Singh and Rachie, 1985). Further, cowpea contains 6.3% fiber, 0.0074% Thiamine, 0.0042% Riboflavin and 0.00281% Niacin, and rich is in lysine and tryptophan (Purseglove, 1995). These nutrients will to a large extent satisfy the ingredients required of a bacteriological medium (Young, 1961; Harper, 1993). This composition suggests that cowpea, when purposefully processed, could be used as a possible non-synthetic microbial medium that may be stored in a powdered form.

Aim

It was the aim of this study to prepare a general purpose solid Cowpea Agar (CPA) medium, use it to culture selected bacteria and compare the growth and cultural characteristics of these selected bacteria on the CPA to that on standard Nutrient Agar (NA).

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Table 1. Colonial growth characteristics of selected bacteria on Nutrient and unfermented Cowpea Agars.

Bacteria / Feature	*K. pneumoniae*			*S. aureus*			*E. coli*			*E. faecalis*			*S. epidermidis*			*S. typhi*		
	NA	CPA $_{U20}$	CPA_{U40}	NA	CPA $_{U20}$	CPA_{U40}	NA	CPA $_{U20}$	CPA_{U40}	NA	CPA $_{U20}$	CPA_{U40}	NA	CPA $_{U20}$	CPA_{U40}	NA	CPA $_{U20}$	CPA_{U40}
Shape	C	C	C	I	I	I	C	C	C	C	C	C	I	I	I	ng	ng	ng
Opacity	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	ng	ng	ng
Colour	W	W	W	M	M	M	Cr	M	M	M	M	M	Cr	Cr	Cr	ng	ng	ng
Elevation	R	R	R	R	R	R	F	C_O	C_O	C_O	C_O	C_O	C_O	C_O	C_O	ng	ng	ng
Surface	S	S	S	Du	D_U	D_U	S	S	S	S	S	S	S	D_U	D_U	ng	ng	ng
Odour	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	ng	ng	ng
Consistency	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	ng	ng	ng
Edge	E	E	E	D e	De	Rh	E	E	E	E	E	E	E	Rh	Rh	ng	ng	ng

NA = Nutrient Agar; CPA $(ADD_U)_{20}$ = 20 g Cowpea Agar; CPA $(ADD_U)_{40}$ = 40 g Cowpea Agar; C = circular; C_O = Convex; Cr = Cream; De = Dentate; D_U = Dull; E =Entire; F = Flat; I = Irregular; M = Milky; O = Opaque; P = Present; R = Raised; Rh = Rhizoid; S = Smooth; V = Viscid; W = White; ng = no growth

MATERIALS AND METHODS

Preparation of media

One kg of black-eyed cowpea was washed with ordinary tap water and immediately cooked in distilled water for 2 h. The cooked cowpea was spread as a single layer on a white paper sheet and dried in a Gallenkamp incubator at 60^0C for 4 days to attain a constant weight. The dried cowpeas were pulverized with a hammer mill and sieved; first, with a laboratory sieve of aperture size 2 mm (Endecott, London) to remove the 'eyes' of the pea, and again with another sieve with aperture size 0.2 mm (Endecott, London) to obtain cowpea powder. The cowpea powder was stored in black polythene bags at 4^0C till use. This cowpea powder was thus an unfermented one. Another 1 kg of cowpea was first steeped in water for 24 h to ferment before being cooked, pulverized, sieved and stored at 4^0C prior to use as described above. To either 20 g or 40 g of fermented or unfermented cowpea powder, 15 g agar (Agar Agar, Reidel-de-Maën, Germany) was added and the volume made up to one litre with distilled water. These were designated as $CPA_{U20,}$ CPA_{U40} (ie unfermented CPA) and $CPA_{F20,}$ CPA_{F40} (that is, fermented CPA). The pH of the cowpea agars were determined by the microprocessor pH meter (Jenway).

Nutrient Agar (MERCK, Darmstadt, Germany) was prepared by weighing 20 g into one liter of distilled water. All the media were sterilized at 121^0C for 15 min before being poured into Petri dishes for plating of selected test bacteria. The CPAs had to be shaken during pouring to ensure uniform mixture.

Culture of selected bacteria

The following test bacteria were obtained from University of Ghana Medical School and TOPP Laboratories: *Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli, Enterobacter faecalis, Staphylococcus epidermidis* and *Salmonella typhi.* These bacteria were rejuvenated in nutrient broth (MERCK, Darmstadt, Germany) at 37^0C for 24 h. From these stock cultures, 10-fold serial dilutions were made in peptone water (MERCK, Darmstadt, Germany). From these serial dilutions, 0.1 ml was used for surface plating on the media in quadruplates. The plates were incubated at 37^0C for 24 h. Culturing on NA was for control and comparison. Thereafter, bacterial populations in were counted and expressed as Colony Forming Units (cfu). Colonial characteristics were also observed.

RESULTS AND DISCUSSION

The pH of the unfermented cowpea agars was 6.7 and for the fermented cowpea agars, 3.9. All the cowpea agars were chocolate-brown in colour, odourless and of firm texture.

The fermentation of the cowpea was expected to produce simple nutritional substances that could be utilized by the test organisms. Unfortunately, all the test organisms did not grow on the CPA made from fermented cowpea (that is $CPA_{F20,}$ CPA_{F40}). It is suggested that the fermentation produced organic acids that had cidal effect on the test organisms. Cuk et al. (1987) had demonstrated that organic acids account for the cidal effect on pathogenic bacteria. Table 1 shows that all test organisms, except *S. typhi,* grew on the unfermented CPA (CPA_{U20} and CPA_{U40}) and NA. The fastidious growth requirements of *S. typhi* are so well known that its inability to grow on such ordinary media was not unexpected.

Table 1 also shows that there were no differences in colonial characteristics for *K. pneumonia* on NA, CPA_{U20} and CPA_{U40}. For *S. aureus* the only colonial difference was the nature of the edges. Whereas colonies on NA and CPA_{U20}, were dentate, they differed from those on $CPA_{U40,}$ which was rather rhizoid. Colonial characteristics of *E. coli* differed only in elevation. Colonies of *E. coli* were flat on NA but convex on

Table 2. Colony forming unit populations of selected bacteria on nutrient and cowpea agars (in cfu).

Bacteria / Dilutions	*K. pneumoniae*			*S. aureus*			*E. coli*			*E. faecalis*			*S. epidermidis*			*S. typhi*		
	NA	CPA $_{U20}$	CPA$_{U40}$	NA	CPA $_{U20}$	CPA$_{U40}$	NA	CPA $_{U20}$	CPA$_{U40}$	NA	CPA $_{U20}$	CPA$_{U40}$	NA	CPA $_{U20}$	CPA$_{U40}$	NA	CPA $_{U20}$	CPA$_{U40}$
10^1	nd	TNTC	TNTC	nd	TNTC	TNTC	nd	TNTC	TNTC	nd	TNTC	TNTC	nd	TNTC	TNTC	ng	ng	ng
10^2	$2.4x10^5$	$4.8x10^5$	$2.25x10^5$	TNTC	TNTC	TNTC	TNTC	$2.04x10^4$	$2x10^4$	$1.92x10^4$	$3.5x10^4$	$2.46x10^4$	TNTC	TNTC	TNTC	ng	ng	ng
10^3	$4x10^5$	nd	$1x10^6$	$4.05x10^5$	nd	TNTC	TNTC	$1.66x10^5$	$1.18x10^5$	$1x10^6$	$5x10^4$	nd	TNTC	nd	TNTC	ng	ng	ng

TNTC = Too numerous to count, that is, >300 TC colonies at the dilution. nd = not done; ng = no growth.

CPA$_{U20}$ and CPA$_{U40}$. Colonial characteristics of *E. faecalis* were identical for CPA$_{U20,}$ CPA$_{U40}$ and NA.The surface of the colonies of *S. epidermidis* was smooth and reflecting on NA but rather dull on CPA$_{U20}$ and CPA$_{U40}$. Further, the edges of colonies of *E. faecalis* were entire on NA and rhizoid on CPA$_{U20}$ and CPA$_{U40}$.

From Table 2, the bacterial populations on CPA$_{U20}$ were greater than on CPA$_{U40}$ at the same dilutions. It is suggested that the greater the content of cowpea powder in the medium the greater the amount of antinutritional factors such as trypsin and protease inhibitors. These will lower the nutrients available to the bacteria (Bressani, 1985), though having subjected the cowpea to heating by way of boiling and drying at 60^0C, trypsin inhibitory activity would have reduced to 80-90% (Della Gatta et al., 1989; Bressani et al., 1982). At the same dilutions, we also notice that the colonial units on CPA$_{U20}$ were comparatively more than on NA for *K. pneumonia* and *E. faecalis. E. coli* grew more buoyantly on NA than on CPA$_{U40}$. For *S. aureus* and *S. epidermidis* the colonial populations at the dilution of investigation were too many to count on CPA$_{U20,}$ CPA$_{U40}$ and NA.

Storage of the cowpea powder for at least three months did not reduce the shelf life of the cowpea powder.

The cost implication of the use of NA and CPA is that whereas it costs $0.02 to prepare 1 L of CPA$_{20}$, it costs $1.60 to prepare the same quantity of NA.

Conclusions

Generally CPAs of this study had more CFU of the test organisms growing on them than on NA. The use of 20 g of unfermented cowpea in preparing CPA was optimum. The use of cowpea to prepare a general purpose solid medium for non-research school use is feasible and also cheaper than commercially produced NA.

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