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Short Communication

Assessment of Native Agar Gels Extracted from *Gracilaria debilis* and *Gracilaria salicornia* Harvested Along the Tanzanian Coast for Culturing Microorganisms

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***Abstract*—** Native agar gels extracted from *Gracilaria debilis* and *G. salicornia* harvested during the rainy and dry seasons, were assessed for culturing the microorganisms *Micrococcus luteus*, *Saccharomyces cerevisiae* and *Pleurotus flabellatus*. Agars extracted from plants harvested during the rainy season were suitable for culturing bacteria, and yeast cells using the 'Pour Plate' method, and for mushroom tissue culture. Agar extracted from *G. debilis* harvested during the dry season could be used for culturing bacterial cells by the 'Spreading and Streaking' method. The potential for laboratory applications and for mushroom spawn production using agar extracted from Tanzanian seaweeds is discussed.

INTRODUCTION

Agar is extracted from species of the red algal genera *Pterocladia*, *Gelidium*, *Gracilaria*, *Phyllophora*, *Ahnfeltia*, *Campylaephora*, *Acanthopeltis* (Lewis *et al*., 1990), *Gelidiella* and *Gracilariopsis* (Armisen, 1995). About 10,000 tonnes of agar are currently produced worldwide. The major producing countries are Japan, Spain, Chile, Mexico, China and the Republic of Korea. Prices of the product depend on gel strength specifications and on the agar application (Critchley, 1997).

Natural-grade agar (native agar), is the first extract from the seaweed and is usually produced in the country where the seaweed is cultivated. This reduces the bulk that needs to be transported, thus reducing costs. At its destination (e.g. Europe and Japan) it is usually processed further to obtain high value gelling agents, such as agarose. Currently there is a shortage of exploitable populations of agar-producing seaweeds (agarophytes), and high quality agars are consequently expensive products.

Tanzania has large quantities of natural stocks of the commercially exploitable agarophyte algae, such as species of *Gracilaria* (see Jaasund, 1979). The most abundant species, *G. debilis* (previously known as *G. crassa*, Buriyo *et al*., 2004), *G. fergusonii* and *G. salicornia* have been reported to have a potential for agar production (Semesi, 1987; Buriyo, 1999), however, these are not currently exploited. This is partly due to lack of information on their suitability (and demand) for the production of good quality native agar.

Currently, microbiological work in Tanzania uses imported agar, which costs about US$ 300 per kg. The use of locally produced natural-grade agar is likely to be more cost effective and save foreign exchange. In addition, the local production of natural grade phycolloids will promote the use of Tanzanian agar in other markets.

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This study therefore, investigated the suitability of native and alkali-modified agar gels extracted from *G. debilis* and *G. salicornia* from Tanzania for culturing bacteria, yeast and fungi using different methods.

MATERIALS AND METHODS

Plant collection and agar extraction

The algae were collected during the wet and dry seasons, from Oyster Bay in Dar es Salaam and Chwaka Bay in Zanzibar. In the laboratory, algal samples were sorted, identified using Jaasund (1979) and other relevant taxonomic literature (Buriyo, 2006) and pooled in respective taxa. Plants for agar extraction were rinsed with tap water, placed in plastic trays and dried in the sun. Sun-dried samples were further dried at 60°C in the oven until a constant weight was reached before agar extraction. Agar gel extraction was done according to the method described by Semesi (1979) and modified by Buriyo (1999).

Test microorganisms and media

A yeast strain, *Saccharomyces cereviciae*, a bacterial strain, *Micrococcus luteus*, and a local edible mushroom strain, *Pleurotus flabellatus*, were used as cultivars on the agar preparations. The test strains were obtained from the culture collection of the Applied Microbiology Unit, University of Dar es Salaam. Nutrient agar (NA) and Malt Extract agar (MEA) were prepared by using 1.5 % (w/v) of native agar from *Gracilaria* on plates according to the Oxoid Manual (1995). All experiments used 1.5 % (w/v) of standard agar (Oxoid Bacto Agar) as a control.

Culturing

Overnight cultures of *M. luteus* and *S. cereviciae* were used to inoculate NA and MEA plates, respectively, by streaking for single colonies, and for colony counting by the 'spread' plate and 'pour plate' methods. For the spread plate method, serial dilutions of up to 10^{-7} were made and aliquots (0.1 ml) of the 10^{-6} and 10^{-7} dilutions were then spread, in duplicate, on surface of dry plates using a sterile Drigalsky spatula. For the pour plate method, 0.5 ml aliquots of overnight cultures were mixed with 9 ml of molten agar and poured onto sterile plates. The inoculated plates were incubated at 25, 30, and 37°C for 16-48 hours. Growth of colonies was monitored and the media was assessed by observing the segregation of colonies together with their appearance and shape.

Tissue culture of *P. flabellatus* was carried out according to Stamets and Chilton (1983) on MEA plates with native and commercial agar. The plates were incubated at 30°C and monitored for growth of mycelia, and the time taken for mycelia to fully colonize the medium was recorded.

Gel strength

The strength or firmness of the gels was determined using an apparatus designed and manufactured by INNOVEST RONANG SWEDEN as described by Buriyo (2006).

RESULTS AND DISCUSSION

Quality of nutrient agar media

Native nutrient agar plates prepared from agar extracted from *G. debilis* and *G. salicornia* harvested during the rain season appeared opaque, with a grainy surface, whereas the commercial agar plates were clear with a smooth surface (results not shown). The media were stable on incubation at room temperature (23-25° C), but became soft and watery at the edges when incubated at 30 and 37 °C. This observation may be explained by the low gel strengths (128-240 g/cm^2) for the agar samples tested compared to the commercial agar which had a gel strength of about 490 g/cm^2 (see Buriyo and Kivaisi, 2003).

The medium prepared with native agar from *G. debilis* harvested during the rain season developed a whitish surface layer that could be peeled of after incubation at 30°C (Plate 1). In contrast, the native agar medium prepared from *G. debilis* harvested during the dry season was very clear,

Plate 1. Nutrient agar medium prepared with native agar extracted from *Gracilaria debilis*. Note the turbid layer that can peel off

homogeneous with a smooth surface and appeared similar to commercial NA plates. The improvement may partly be explained by the higher gel strength (251 ± 16 g/cm^2) of the agar samples as reported by Buriyo & Kivaisi (2003).

The occurrence of agar with higher gel strength during the dry period and vice versa has been reported by other workers (Abbott, 1980; Asare, 1980). Low gel strength of agar gels from algal materials harvested during the rain season has been attributed to low salinity which is said to be responsible for the loss of ions including Ca^{2+} and K^+ from the algal cells (Yarish *et al.*, 1980). These ions have been reported to increase gel strengths of some algal polysaccharides such as alginates from brown algae and other gels from red algal species such as *Eucheuma*, *Gracilaria*, *Hypnea* etc. (Grant *et al.*, 1973; Semesi, 1979). Further evidence on the effect of salinity on gel strength was provided by Bird (1988) who reported low gel strength for agar extracted from *Gracilaria* species cultivated at low salinity (17°/$_{oo}$). There was no obvious difference between agar gels prepared from algal materials harvested from Oyster Bay and Chwaka Bay.

Suitability of native agar for culturing microorganisms

A good solid culture medium for microoganisms should allow growth and segregation of single colonies which may be distinguishable by shape and colour. The medium should also remain solid at optimal temperatures for growth of a wide range of microoganisms. Native agar from the studied algal species appeared to have a limited potential for use to culture different microorganisms using different methods (Table 1). Media prepared by using agar extracted from algae (both species) harvested during the rain season allowed segregation of colonies of *Saccharomyces cereviciae* (Plate 2) and *Micrococcus luteus* (Plate not available) by only the pour plate method, at incubation temperatures

Table 1. Appearance of colonies of test microorganisms on native agar media using various methods and incubation temperatures

Organism	Medium	Test methods	
		Streaking (30,37° C)	**Pour plate (23- 25° C)**
Micrococcus luteus	*G. debilis*[b]: NA-native	Well segregated colonies	ND
	G. debilis[a]: NA-alkali treated	Well segregated colonies	Single colonies
	G. salicornia[a]: NA-native	Spread and joined colonies	ND
	G. salicornia[a]: NA-alkali treated	Spread and joined colonies	ND
Saccharomyces cereviciae	*G. debilis*[a]: MEA-native	Spread and joined colonies	Well segregated colonies
	G. debilis[a]: MEA-alkali treated	Segregated colonies	ND
	G. salicornia[a] : MEA-native	Spread and joined colonies	Well segregated colonies
	G. salicornia[a] : MEA-alkali treated	Segregated colonies	Well segregated colonies

ND = not determined; [a] = rain season harvest; [b] = dry season harvest

Plate 2. *Saccharomyces cereviciae* growing on *G. salicornia* native agar medium (Pour plate method was used)

samples) and fully colonised the plates within five days as it did on the commercial medium at 23, 30 and 37°C. The mycelia appeared white and fluffy similar to the control medium. This finding is important to mushroom research laboratories and to mushroom growers who could use local native agar for preparation of mushroom spawn. Future studies should aim at optimizing the quality of native agar.

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Plate 3. *Pleurotus flabellatus* mycelia growing on commercial MEA (right) and native MEA (left)

below 30°C. At higher temperatures, merging of adjacent colonies was observed. This was because of the softening of the agar and formation of a watery surface which spread cells widely over the plates. In contrast, the native agar medium prepared from *G. debilis* harvested during the dry season allowed streaking of bacterial cells to form single colonies. Agar from *G. salicornia* harvested during the dry season was not tested. Again the difference in firmness of the agar gels from algal material harvested during the rain and dry seasons can be explained by the differences in gel strengths. As far as use of native agar for culturing microorganisms is concerned, there appears to be no documented studies for comparison. The suitability of the native agar for culturing mushroom mycelia is illustrated in Plate 3. *Pleurotus flabellatus* mycelia grew very well on native MEA (prepared from all agar

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