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Coconut based in-house developed medium for fungal growth

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By dextrose and protein content and p^H level, tender coconut is almost identical with Sabouraud's Dextrose broth. The study is to evaluate the suitability of agar based solid medium prepared with coconut water for growth of fungi. As coconut milk contains various fatty acids, lipo-philic fungus will also be tested in its solid version. Reference strains of *A. flavus*, and *A. niger*, along with their clinical isolates and also clinical isolates of *T. rubrum, H.capsulatum, N. brasiliensis* were grown into in-house prepared coconut agar medium and SDA medium. Using standard inoculum quantitative estimations were done for growth of reference strain of *C. albicans* in both media. Clinical isolates of *Malassezia furfur* and scraping materials of Pityriasis versicolor cases were inoculated into coconut milk agar along with conventional method of culture. Growths were compared. The growths in coconut water agar were almost similar with growths in SDA for all tested fungi. *M. furfur* grew well in coconut milk agar without oil overlay. Coconut based solid media can be cheap alternative of SDA. Coconut milk agar can be good subsitute for Dixon's agar for growth of *M. furfur.*

Key words : Culture medium, *Malassezia* sp. *coconut water*

INTRODUCTION

Coconut water, a natural source of sterile fluid contains considerable amount of sugars and other nutrients (Gopalan *et al.*, 1989a) in different stages of maturation of coconut (*Cocos nucifera*). The composition and pH of tender coconut water (approximately 5) is comparable to that of conventional Sabouraud's Dextrose broth. It contains approximately 1.5-5.5% sugars mainly in the form of glucose and fructose, protein 0.9%, mainly in the form of essential amino acids, fat and fatty acids 1.4%, sufficient electrolytes and vitamins. In the present study, our aim is to see the growth of different fungi in tender coconut water as well as in coconut agar medium and to compare the results with growth of those fungi, in conventional liquid and solid media. Lipophilic fungi like *Malassezia* sp. is usually grown in Sabouraud's Dextrose Agar (SDA) with olive oil overlay. The coconut milk obtained from the juice of the copra has high oil content mainly composed of various fatty acids including oleic acid. This may promote the growth of lipophilic fungi like *Malassezia furfur.* So an agar based solid medium using coconut milk, has been tried for growth of these fungi without need of any extra oil on surface.

MATERIALS AND METHODS

An aliquot of 1.5 × 10^6/ml saline suspension of reference strain *Candida albicans (MTCC 227)* was prepared and 20 µl of the suspension was inoculated into each of 6 tubes containing 1 ml of SD broth in one and 1 ml tender coconut water in other 5 tubes for 5 different lots of coconut. Both the broths were incubated at 25ºC for 18 hrs. Then from each tube 20 µl of broth cultures were inoculated into SDA plates. Plates were incubated at 25ºC for 24 hrs. and were examined for comparative growths in the two types of broth by colony counting. Five repetitions of the test were done.

Reference strains of *Aspergillus flavus (MTCC 760279)* and *Aspergillus niger (MTCC 1344)* and five clinical isolates of *Trichophyton rubrum*, one *Histoplasma capsulatum* and one *Nocardia brasiliensis* were inoculated both in SDA and coconut agar medium, then incubated at 25ºC up to 4 weeks. The growths in paired media were

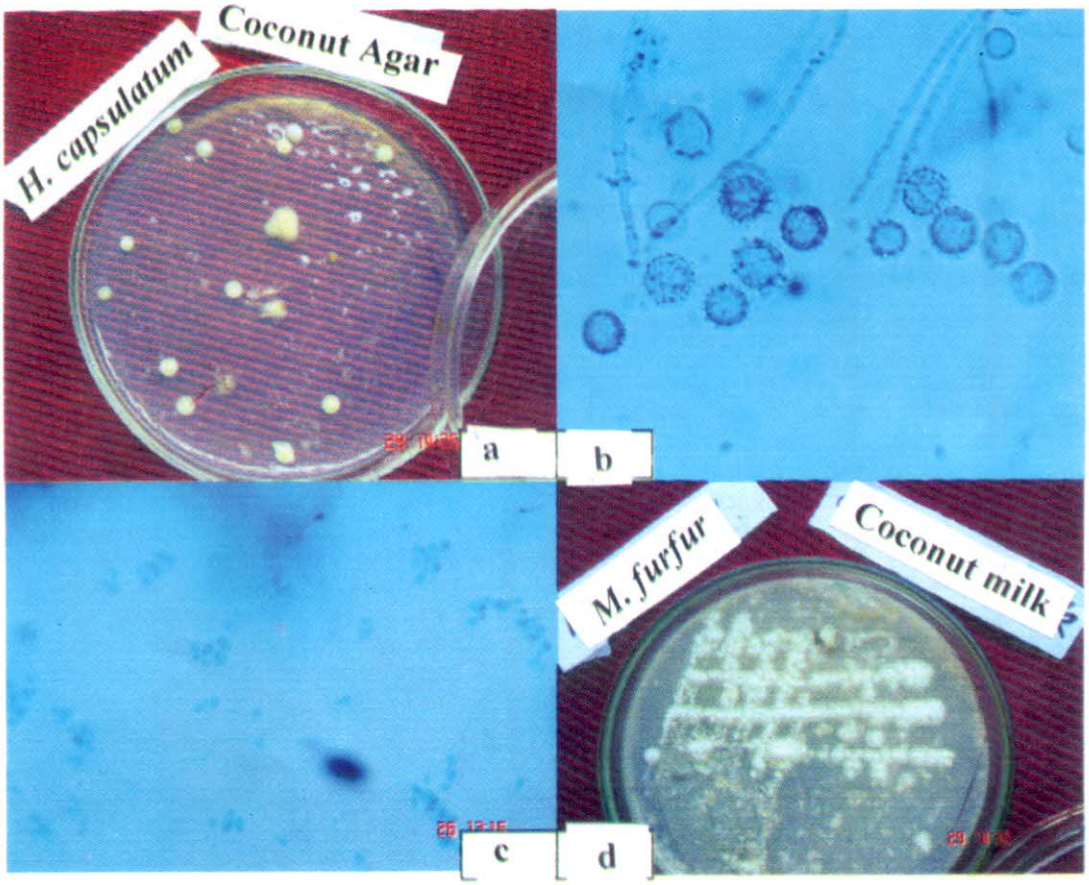

Fig. 1 : (a) Colony and (b) morphology [400x] of *H. capsulatum* grown in coconut ; agar medium (c) Morphology [400x] & (d) colony of *M. furfur* grown in coconut milk agar medium.

examined and compared by eye estimation in three grades e.g. higher, lower and equivocal growth. Lactophenol cotton blud (LCB) mounts of both growths were also examined.

Ten clinical isolated of *Malassezia furfur* were taken and inoculated into three media : coconut milk agar, modified Dixon's agar and SDA overlaid with olive oil. All the plates were incubated at 25ºC and examined daily for two weeks. Once growth appeared the growths in different media were compared. Fungal morphology was examined from LCB mounted smears. Skin scales from lesions of ten patients with suspected Pityriasis versicolor, that were confirmed by direct microscopic examinations, were tested. Samples were inoculated into the coconut milk agar medium and examined for growth supportive evidence two weeks after incubation at 25ºC.

RESULTS AND DISCUSSION

The quantitative estimation of the growths of reference strain *C. albicans* showed (Table 1) almost similar growths in SD broth and tender coconut water, without much variations in lot differences (by range and average number of colony in each lot coconut water). Evaluation of the growth of reference strain *Aspergillus flavus, Aspergillus niger* and clilnical isolates of *Trichophyton rubrum* and *Histoplasma capsulatum, Nocardia brasiliensis* revealed that the coconut agar medium supported the growth of these organisms, though the growths were slightly lower than that in the conventional media, by eye estimation. Two factors that favored

Table 1 : Colony count of *C.albicans* in different sets of test media.

Repetition	SD broth	Tender Coconut Water				
		Lot-1	Lot-2	Lot-3	Lot-4	Lot-5
1st.	72	69	68	70	65	72
2nd.	68	62	59	61	65	67
3rd.	66	71	58	63	73	59
4th.	70	70	72	74	72	69
5th.	65	64	73	71	60	63
Total	341	336	330	339	335	330
Average	68	67	66	68	67	66

the growth of these fungi in coconut agar medium are the acidic pH and the content of optimum carbon and nitrogen sources. The composition of the test medium was very much sililar to that of SDA medium.

Microscopic examination of the lactophenol cotton blud (LCB) mounted preparations from Reference strains of *Aspergillus flavus (MTCC 760279)* and *Aspergillus niger (MTCC 1344)* and *Histoplasma capsulatum* (clinical isolate) growths revealed that the conidiogenesis occurred more in the coconut agar medium Fig. 1, a & b) as compared to the conventional media. The coconut agar medium seemed to be a better sporulating medium as compared to the SDA medium though growths are slightly slower.

Malassezia furfur is a lipophilic organism having an absolute requirement for lipids for their growth (Panja, 1927 ; Midgely *et al.,* 1998). Our results showed that the growth of the *Malassezia* species was much higher in the coconut milk agar medium as compared to the growth in commonly used SDA medium with olive oil overlay or the modified Dixon's agar medium. The latter had oleic acid incorporated into the medium (Leeming and Notman, 1987) and the same was also sufficiently present in coconut milk. (Gopalan *et al.,* 1989b). Good growths of *M. furfur* in coconut-milk agar from clinical materials (Fig. 1, c & d) of all microscopically confirmed pityriasis versicolor cases also supported the superiority of this in-house developed medium.

The coconut milk has a high cotent of coconut oil that is composed of a number of fatty acids including oleic acid that provides the organism with the fatty acid required for its growth. Thus the cumbersome procedure of overlaying oil on inoculated SDA plates can be avoided which also lowers the chances of contamination due to oil overlay. Moreover, the oil overlaid plates are not suitable for performing the anti-fungal sensitivity tests for which we have to depend on the modified Dixon's agar which has oil incorporated into the medium and preparation of which requireds a number of expensive ingredients. Thus the coconut milk agar medium that contains most ingredients from natural sources, can be a better alternative to the conventional media for isolation of the *Malassezia* sp. and also for the performance of the anti-fungal sensitivity tests. The preparation of the medium is easy and is also inexpensive making it a very cost-effective. The utility of this in-house developed medium has to be tested with more number of clinical materials in future.

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