ORIGINAL RESEARCH

Use of Sunflower Seed Husk Agar for Differentiation of *Candida albicans* and *Candida dubliniensis*

Swati Raghunath Sapkal[1], Shilpa R. Shah[2], Shubhangi Gadgil[3], S. A. Kulkarni[4]

ABSTRACT

Introduction: *Candida albicans* is the commonest species of Candida causing infections. A new recently described candida species named *Candida dubliniensis* is phylogenically closely related to *Candida albicans* and known to cause infections. It has many similarities with *Candida albicans* in morphological and physiological characteristics such as positive germ tube test, similar biochemical patterns and also formation of chlamydospores in rice extract agar and corn meal agar. Such close proximity in variety of features between the two species has led to confusion and misidentification of isolates of *Candida dubliniensis* as *Candida albicans* in clinical laboratories.

Material and Methods: In our study we report that sunflower seed husk agar can be used to differentiate *Candida albicans* and *Candida dubliniensis*. In total of 100 confirmed clinical isolates of Candida obtained from various clinical specimens and identified by standard methods, 47 showed positive germ tube test and chlamydospore formation on corn meal agar (CMA). All these isolates were subcultured on Sunflower seed husk agar (SSHA) and their growth at 45°C was noted on Sabouraud's dextrose agar(SDA).

Results: On SSHA 6 (100%) *C. dubliniensis* produced rough colonies and 39 (95.12%) *C. albicans* produced smooth colonies and 2(4.87%) produced rough colonies.41(100%) *C. albicans* showed growth at 45°C on SDA and none of *C.dubliniensis* showed growth at 45°C on SDA.

Conclusion: All the isolates of *C.dubliniensis* (100%) produced rough colonies and abundant chlamydospores on SSHA agar. 95.12% isolates of *C. albicans* showed smooth colonies with lack of chlamydospore formation on SSHA. This suggests that SSHA can be a simple alternative method for presumptive differentiation of *C.dubliniensis* from *C. albicans*. This medium is cheaper and can be prepared with easily available ingredients with simple composition. Hence we feel that SSHA can be an ideal medium for routine use in clinical microbiology laboratory.

Keywords: Sunflower Seed Husk Agar (SSHA), *C.dubliniensis*, *C. albicans*, Rough Colonies, Chlamydospores

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INTRODUCTION

Fungi are one of the important pathogen causing human infections. Amongst fungi, the genus Candida are the most pathogenic.[1] Candida species forms a part of normal flora. Over the past few decades, the number of opportunistic infections caused by Candida species are on rise due to variety of factors like indiscriminate use of antibiotics, indwelling intravascular catheters, cytotoxic therapies, immunosuppressive therapies, immunological disorders and recent increase in the no. of AIDS patients.[2,3]

Candidiasis has gained much clinical significance in the recent years due to variety of candida species causing infections which differ in expression of putative virulence factors and antifungal susceptibility.[3] Hence identification of candida isolates up to species level has gained a lot of importance in view of potential clinical significance.

C.dubliniensis phylogenically closely resembles *C. albicans.*[4] It is a recently described species initially isolated from cases of recurrent candidiasis from patients of human immunodeficiency virus (HIV) infection.[5,7] It also shares many morphological and physiological characteristics with *C. albicans*, such as positive germ tube test, similar biochemical reactions and formation of chlamydospores on rice extract agar and corn meal agar etc. These similar features between the two species led to the misidentification of isolates of *C.dubliniensis* as *C. albicans.*[5-8]

In our study we report that sunflower seed husk agar can be used to differentiate *C. albicans* and *C.dubliniensis.*

MATERIAL AND METHODS-

A total of 100 confirmed clinical isolates of Candida obtained from various clinical specimens like urine, blood, sputum, body fluids, vaginal swab, throat swab etc. from June 2013 to July 2014 were included in the study. This study was conducted after approval of Institutional Ethical Committee.

The isolates were first subcultured on CHROM agar Candida plates and incubated at 37℃ for 24 hours aerobically to check for purity and identification.

All isolates were identified based on standard mycological methods, germ tube production, morphology on Corn meal agar, color on CHROM agar, sugar assimilation and fermentation and growth at 45°C. The germ tube positive isolates were inoculated on Sunflower seed husk agar for differentiation of *C. albicans* and *C.dubliniensis* on the basis of colony morphology and chlamydospore production.

For preparation of Sunflower seed husk agar, husk of sunflower was separated manually. 50 gm sunflower seed husk was pulverized in domestic grinder for 3-4 min. The pulverized husk was boiled for 30 minutes with 1 liter of distilled water and filtered through several layers of gauze. 10 gm glucose was added in filtrate. The volume was made up to 1 lit. and P^H adjusted to 5.5 before autoclaving at 15 pounds pressure for 15 min.[4]

On SSHA two streaks of 1.5 cm were made at approximately one cm apart with sterile inoculating needle without piercing into the medium. The needle is flamed again to become red hot, cooled and a 'S' shaped streak was made across two streak marks. This plate was incubated at 28°C. It is then observed for colony morphology and examined under microscope for chlamydospore formation.

All *C. dubliniensis* isolates produce rough colonies with hyphal fringes and abundant chlamydospore production while *C. albicans* produce smooth colonies and no chlamydospores. All the germ tube positive isolates were streaked on SDA incubated for 24-48 hours at 45°C. All *C. albicans* strains showed growth at 45°C, while *C.dubliniensis* did not show growth at 45°C.

RESULT

Out of total 100 isolates, 41 were *C. albicans* and 6 were *C. dubliniensis* and remaining were other Candida species.

Out of total 100 Candida isolates on CHROM agar, 41 isolates showed light green color indicating *C. albicans* species and 6 isolates showed dark green color indicating *C.dubliniensis.* These were further subjected to study chlamydospore production on SSHA and growth at 45℃ on SDA.

Out of 41 *C. albicans* isolates 95.12% strains showed smooth colonies and 4.87 % strains showed rough colonies on SSHA and none of the strain showed chlamydospores on SSHA. Out of 6 isolates of *C.dubliniensis* 100% isolates showed

Species	Total isolates (%)
C. albicans	41 (41)
C. tropicalis	35 (35)
C. glagrata	14 (14)
C. dubliniensis	06 (06)
C. parapsilosis	03 (03)
C.guillermondii	01 (01)
Total	100 (100)

Table-1: Different species of Candida isolates on CHROM agar

Characters	*C. albicans* **(41)**	*C.dubliniensis***(6)**
Smooth growth on SSHA	39 (95.12%)	0
Rough growth on SSHA	2 (4.87%)	6 (100%)
Chlamydospores on SSHA	0	6 (100%)
Growth at 45℃	41 (100%)	0

Table-2: Observation of colony morphology and chlamydospore production on SSHA and growth at 45℃ on SDA by *C. albicans* and *C.dubliniensis*

Figure-1: Growth of *C.dubliniensis* on CHROM agar (dark green) B – Growth of *C. albicans* on CHROM agar (Faint green)

Figure-2: Rough colony of *Candida dubliniensis* (A) and smooth colony of *Candida albicans* (B)on sunflower seed husk agar

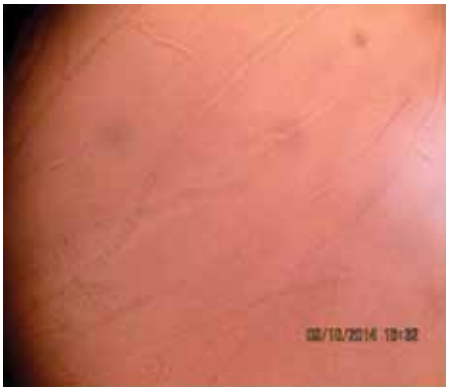

Figure-3: *Candida albicans* on SSHA at 40X

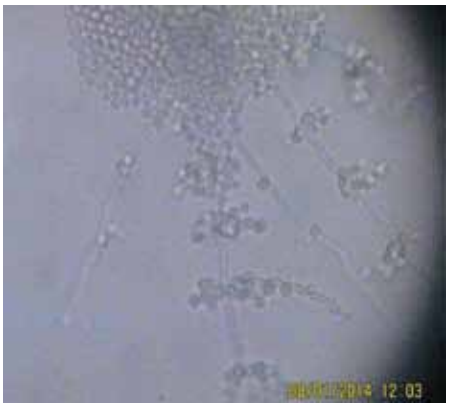

Figure-4: *Candida dubliniensis* on SSHA at 40X

Figure-5: No growth (no.1) of *Candida dubliniensis* at 45℃ and growth (no.2 to 8) of *Candida albicans* at 45℃

rough colonies and 100% isolates showed chlamydospores on SSHA.100% isolates of *C. albicans* showed growth at 45℃ while *C.dubliniensis* did not show growth at 45℃. All these isolates were further confirmed by VITEC 2 YST as *C. albicans*(41) and *C.dubliniensis*(6) respectively.

DISCUSSION

In clinical laboratories the primary test used for differentiation of *C. albicans* and non albicans strains is germ tube formation. The two candida species *C. albicans* and *C.dubliniensis* both give positive germ tube test, hence further differentiation is necessary. In our study we have studied use of a simplified medium sunflower husk seed agar for differentiation of *C.dubliniensis* from *C. albicans. C.dublinensis* shows rough colonies and presence of chlamydospores and *C. albicans* shows smooth colonies and absence of chlamydospores on SSHA.

Several investigators have focused attention to develop simple and inexpensive methods for discriminating these two species in routine, which include production of rough colonies and chlamydospores on Pal's agar[7], tobacco agar[9], absence of growth on xylose-based agar medium[5], Inability to grow at 45℃[10, 12] and production of dark green colonies on CHROM agar[11] by *C. dubliniensis* isolates.

In our study 95.12% *C. albicans* showed smooth colonies on SSHA and 4.87% grew as rough colonies on SSHA. Whereas 100% *C.dublineinsis* produce rough colonies. This correlates well with study of Asmaa Mosaid etal[7] and Z U Khan etal[4], where 100% *C. albicans* grew as smooth colonies, 97.7% *C.dublineinsis* grew as rough colonies, 100% *C.dublineinsis* showed rough colonies and 96% *C. albicans* showed smooth colonies respectively.

In our study 100% *C.dublineinsis* produced chlamydospore and none of *C. albicans* produced chlamydospore on SSHA. This correlates with study of Z U Khan etal[4] and Aasma Mosiad etal[7]. In our study 100% *C. albicans* showed growth at 45℃ and none of *C.dublineinsis* isolate was grown at 45℃. This is well correlated with findings of Emmunelle Pinann et al[12].

Although the number of *Candida albicans* and *Candida dubliniensis* species in our study is limited ,the results provide an idea about the use of SSHA for their differentiation.

CONCLUSION

All 100% isolates of *C.dubliniensis* produced rough colonies and abundant chlamydospores on SSHA agar. Total 95.12% isolates of *C. albicans* showed smooth colonies with lack of chlamydospore formation on SSHA. This suggests that SSHA can be a simple alternative method for presumptive differentiation of *C.dubliniensis* from *C. albicans*. This medium is cheaper and can be prepared with easily available ingredients with simple composition. Hence we feel that SSHA can be an ideal medium for routine use in clinical microbiology laboratory for differentiation of *C.dubliniensis* from *C. albicans*, which further helps in proper choice of antifungal drugs and cure of patient.

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