IJCRR
Vol 04 issue 23
Section: General Sciences
Category: Research
Received on: 14/10/12
Revised on: 27/10/12
Accepted on: 07/11/12

EVALUATION OF HICROME AGAR – CANDIDA, A NEW DIFFERENTIAL MEDIUM FOR ISOLATION OF CANDIDA SPECIES FROM ORAL TRUSH IN HIV SEROPOSITIVE PATIENTS

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ABSTRACT

Background of study: Oral candidiasis is the most common opportunistic infection in HIV seropositive patients, also predictive of immunosuppression. Though Candida albicans is the predominant isolate in oral candidiasis, there is rise in non albicans Candida infection coupled with high levels of antifungal resistance. There is urgent need for rapid, simple and reliable method to identify yeast isolates. Hicrome agar- Candida is a selective and differential medium for identification of yeasts directly from clinical samples. This medium allows selective isolation of yeasts and simultaneously identifies certain species of Candida.

Aim / Objective: To evaluate the utility of Hicrome agar-Candida in identification of C. albicans and non albicans Candida.

Research Methodology: Two oral swabs obtained from 100 HIV seropositive patients having oral candidiasis were subjected to identification and characterization by standard conventional methods. Simultaneously direct inoculation was done on Hicrome agar- Candida plate.

Conclusion: Of the total 100 samples 103 species were obtained. C. tropicalis was the most common species isolated followed by C. guilliermondi, C. parapsilosis, C. kefyr, C. albicans, C. krusei, C. glabrata, C. fomata and C. pelliculosa. Hicrome agar showed selective growth of all Candida species with distinguishing color for each species. C. tropicalis showed blue color with sensitivity (68%) and specificity (98.72%). C. albicans showed green colored colonies with 100% sensitivity and specificity respectively. C. kefyr showed pink color with sensitivity (61%) and specificity (92%). C. guilliermondi showed 95% sensitivity and 59% specificity. Hicrome agar differentiated mixed culture with all samples.

Keywords: Hicrome agar-Candida, C. albicans, C. tropicalis.

INTRODUCTION

Oropharyngeal candidiasis continues to be a common opportunistic infection in patients infected with Human Immunodeficiency Virus (HIV) and it is the predictive of increasing immunosuppression.[1] Though Candida albicans is the predominant isolate, rise in frequency of isolation of non albicans Candida species is observed. Rapid and reliable identification of these Candida species is essential as they differ in their virulence and sensitivity to antifungal drugs.

Routine identification of Candida species in the clinical microbiology laboratory is based upon the morphological characteristics such as the formation of pseudohyphae and terminal chlamydospores, clusters of blastoconidia at septa when grown on Corn meal agar at room temperature and the formation of germ tube in serum at 37 ^{0}C. In addition, carbon source

assimilation and fermentation tests or commercially available kits are also used as additional diagnostic tests.[2] Despite the availability of these tests, the identification of Candida species is laborious, time consuming and sometimes difficult to interpret. These tests at times may be inadequate or less sensitive and may yield inaccurate identification especially when atypical strains defying classical identification characteristics are encountered.

Mixed growth having C. glabrata, C. krusei, C. parapsilosis and other non albicans Candida are associated with increasing frequency in these patients. There is not only difficulty in their identification, but also clinical therapeutic failure to azoles as these organisms shows increased resistance to azole group of drugs. This is due to selective pressure or increased usage of Fluconazole as prophylactic drug.[3, 4] Although Automated systems are available to accurately identify the isolates to species level and derive their antifungal susceptibility pattern. These automated systems proved to be considerably expensive and are limited to few sophisticated laboratories.

Several CHROMagar-Candida, a chromogen based culture medium has been commercially developed for rapid and reliable identification of C. albicans, as these strains produce β-N-acetylgalactosaminidase enzyme interacting on chromophore substrate incorporated in the media and gives green colored colonies.[3, 5] This media also allows identification of mixed yeast isolates from clinical samples, permitting presumptive identification of C. albicans from other Candida species.

Hicrome agar- Candida (Himedia, Mumbai, India) is one such chromogenic medium employs the same principle and helps in identifying Candida isolates based on colony color and morphology. Hicrome agar identifies C. albicans by imparting green color to the colonies, C. tropicalis shows blue color, C. glabrata showing green-purple color, C. parapsilosis shows pink colored colonies.[5, 6]

Although the manufacturer claim that this media shows better performance with good accuracy in identifying Candida species, there is need to establish its ability in selective isolation and presumptive identification of Candida species, before replacing conventional methods.

Thus the present study was carried out with the objective to prove the utility of Hicrome agar in identification of C. albicans and non albicans Candida in quicker time as compared to identification by conventional methods.

RESEARCH METHODOLOGY

The present study was carried out at the Department of Microbiology, BLDEU'S Sri B M Patil Medical college and Research centre, Bijapur, Karnataka. The study was reviewed and approved by the Institutional Ethical Committee.

Patients were included in the study if they were HIV seropositive irrespective of duration of infection of either sex and of all age group with oral lesion characterized by cream- white, curdy patches or erythematous lesions on dorsum of tongue / buccal mucosa/ pharyngeal wall.[7] Those patients who received antifungal treatment within one month duration were excluded. After taking written informed consent, specimens were collected by firmly swabbing the lesion with two sterile cotton swabs. One swab was used for identification of yeasts by conventional methods and the other swabbed directly on the Hicrome agar plate. Hicrome agar was prepared according to the manufacturer's instructions.

Total 100 swabs showing positive for yeasts on microscopy were subjected to culture on Emmon's modified Sabourauds Dextrose Agar (SDA) supplemented with antibiotics (gentamicin 5µg and chloramphenicol 50µg) and on Hicrome agar plate, incubated at 37^0C. Cream colored pasty yeast colonies on SDA were subjected to Germ Tube Test for two hours, morphology on Corn meal agar (Dalmau Plate Culture method) read

after 48 hours and Auxonographic sugar assimilation test incubated for 7 days for identification of yeasts up to species level.[8] Hicrome agar plates were visualized daily at 24hrs, 72 hrs and followed up to 7 days to check for colonial growth, characteristic color, color intensification and for variation in colony morphology .[6]

Statistical analysis: Parameters like sensitivity (true positive/true positive + false positive), specificity (true negative/true negative+ false positive) were determined.

RESULTS

The study group consists of 100 HIV seropositive patients, comprised 71% male and 29% females. Majority of the patients belong to age group between 31-45 years(50%) followed by age group16- 30 years(26%) and age group 46-60 years(20%). Mean age of the study group is 36 years. In our study, Non albicans Candida was the most common species isolated accounting for 88.35% and Candida albicans accounting for 11.65%. Species distribution is given in table 1.

Out of 100 samples 103 Candida species were obtained. All the isolates showed growth on Hicrome within 24-36 hours, of size 1-3mm and it was difficult to identify Candida species based on color as the exact color and colony morphology was not able to recognize easily within 24-36 hours. Increased Colony size and well differentiated color and colony morphology were well appreciated between 36-48 hours. But in some rare species like C. pelliculosa , C. fomata and one strain of C. tropicalis were observed with well differentiated color after 72 hours, hence there was statistical difference ($P<0.01$) between time of growth and time for intensification of color. Hicrome agar-Candida was able to distinguish green, blue, pink and cream color clearly after incubation up to 48hours. Sensitivity and Specificity of each species is represented in figure 1.

There was absolutely no batch- batch variation, as tested by C. albicans ATCC 90028.

Three mixed cultures having six isolates were identified based on colony color, size and texture. All six isolates were obvious with their characters and they could be easily identified (**table 2**). While on SDA it needed lot of experience to identify mixed growth, except for colony size, there were no other defining variations with different isolates.

There was no significant change in color after 72 hours on further incubation up to 7 days in almost all strains.

DISCUSSION

In the present study, majority of the HIV patients were in the age group of 31-45 years with mean age 36 years with male preponderance accounting for 71% which correlates well with other studies.[9, 10, 11, 12]

Out of 103 Candida isolates obtained in our study, species identification revealed that 91(88.35%) were non albicans Candida, whereas remaining 12 (11.65%) were the C. albicans. In contrast to studies of Lattiff et al.[13] and Enwuru CD et al.[14] who reported 86% and 40.5% of C. albicans respectively. Challocombe SJ et al.[15] and Enwuru CD et al.[14] reported 54% and 59.5% of Non albicans Candida respectively, whereas our study depicted non albicans Candida at much higher prevalence revealing change in trend of infectious agent replacing C. albicans. Perhaps, this may be due to regional differences or selective pressure of antifungal drug usage.

All 12 isolates of C. albicans showed green colonies having sensitivity and specificity 100% which correlated with Odd s et al.[16] Green colored colonies, particularly distinctive for C. albicans were easily identified.

C. tropicalis having sensitivity of 68% goes in agreement with Hiroshi et al.[4] who reported 71%, whereas Odds et al.[16] and Howarth et al.[17] had sensitivity of more than 95%. This sensitivity in our study is less as compared to others.

C. guilliermondi showed 95.72% sensitivity on this medium which is comparable with Hospenthal et al.[6] (96%). Only three isolates of C. parapsilosis showed exact color as described having sensitivity 23.8%, Hospenthal et al.[6] also reported wide range of colors for this species. Many of our isolates showed cream colored colonies with defined morphology instead of pink color which were not included as true positives hence sensitivity of the species reduced in our study. Hence it's not only the color but also the colonial morphology has to be included for accurate interpretation.

C. kefyr and C. krusei had sensitivity of 61% and 44% respectively correlates with the study of Pfaller et al.[18] who reported 66% each. We found it difficult in identifying these species by their colonial morphology as it was showing variation in its appearance except for few which showed typical morphology.

Even the secondary species like C. glabrata and C. pelliculosa showed variation in their shades of color and appearance as in study like Hovrth et al.[17] This was the major limitations of the medium where it was bit less accurate in identifying uncommon species. This may be due to less number of uncommon isolates obtained from our study. This remarks us to carry out further research on secondary species to prove their distinctive character on Hicrome agar- Candida.

Recovery rate found on Hicrome agar was equivalent on SDA in isolation of Candida species. Hicrome agar Candida was slightly superior to the Sabouraud agar in terms of its ability to suppress bacterial contamination. Whereas, the CFU on Hicrome agar were not only less as compared on SDA but also the time taken to show exact growth to be differentiated by color is prolonged with mean duration 39 hours.

Our study found that colonial pigmentation and typical morphology persisted throughout a seven-day period as described by others and the manufacturer of Hicrome agar. Hicrome agar-Candida can readily be applied to identify colonies after the 48 hours of incubation. The present study highlights the fact that, Hicrome agar - Candida differential medium proved to be very effective for identification of four major Candida species i.e. C. albicans, C. tropicalis, C. guilliermondi and C. parapsilosis. As these isolates were readily identified when isolated directly from oral thrush patients in this study.

Its overall superiority has been self-evident in our hands in its ability to reveal mixtures of yeast species present in cultures. Hicrome agar not only gave clue of mixed growth but also alerts to check for mixed growth on SDA.

Routine use of chromogenic media carries the potential for cost savings in the clinical microbiology laboratory. Use of these media could potentially save the time and expense of performing assimilation tests and other fermentative or biochemical testing. In addition, use of CHROM agar Candida can also improve the ability of the mycology laboratory to rapidly identify mixed yeast infections. This capability will also enable clinicians to more rapidly make appropriate antifungal choices, decreasing patient morbidity and mortality.

CONCLUSION

The Hicrome agar- Candida is adequately sensitive to grow most of the important yeasts. C. albicans, C. tropicalis, C. parapsilosis, C. krusei can be identified rapidly and also ability to detect mixed growth shows the usage of this media to higher level. Hicrome agar-Candida can be readily used for selective and differential isolation of Candida species at quicker time.

ACKNOWLEDGEMENT

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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Table I: Species distribution

Sl No	Species	Isolates (n)	Percentage
1	C. albicans	12	11.65%
2	C. tropicalis	25	24.27%
3	C. glabrata	21	20.39%
4	C. parapsilosis	13	12.62%
5	C. kefyr	13	12.62%
6	C.krusei	8	7.77%
7	C. glabrata	6	5.83%
8	C. fomata	3	2.91%
9	C. pelliculosa	1	0.97%
	Total	103	100%

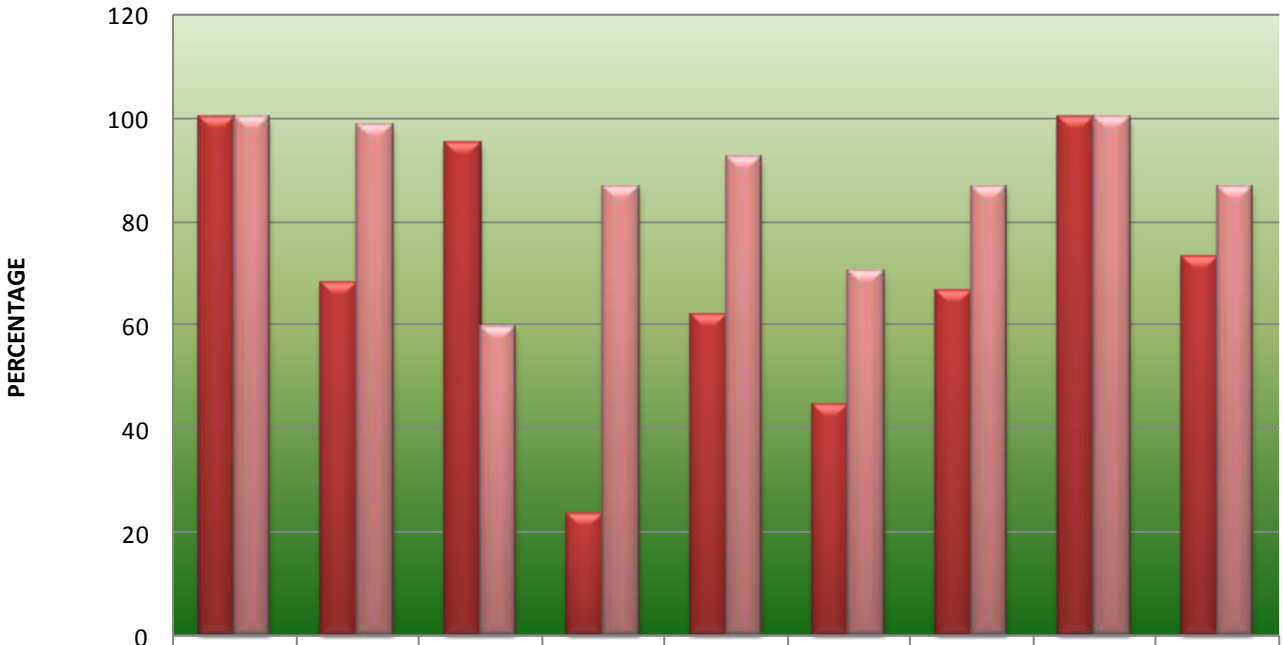
Table I: shows C. albicans 11.65%, among non albicans Candida it is the Candida tropicalis (24.27%) was frequently isolated species followed by Candida guilliermondi (20.39%), Candida parapsilosis (12.62%). Candida kefyr (12.62%), Candida krusei (7.77%) were isolated. Rare species like Candida glabrata (5.83%), Candida fomata (2.91%) and Candida pelliculosa (0.97%) were also isolated.

Table II: Mixed growth

SL NO	SPECIES	On SDA	On Hicrome	COLOR
1	C. guilliermondi + C. tropicalis	2	2	Green, Pink
2	C. tropicalis + C. parapsilosis	1	2	Blue, Cream
3	C. fomata + C. krusei	1	2	Cream, Pink

Table II: shows three mixed cultures identified on Hicrome agar plate with two species each and only one mixed growth identified on SDA plate.

Figure 1: Sensitivity and Specificity of Candida species on Hichrome agar



	C. albicans	C. tropicalis	C. guilliermondi	C. parapsilosis	C. kefyr	C. krusei	C. glabrata	C.fomata	C.pelliculosa
Sensitivity	100	68	95.23	23.08	61.73	44.4	66.67	100	73.21
Specificity	100	98.72	59.75	86.66	92.22	70.21	86.5	100	86.49

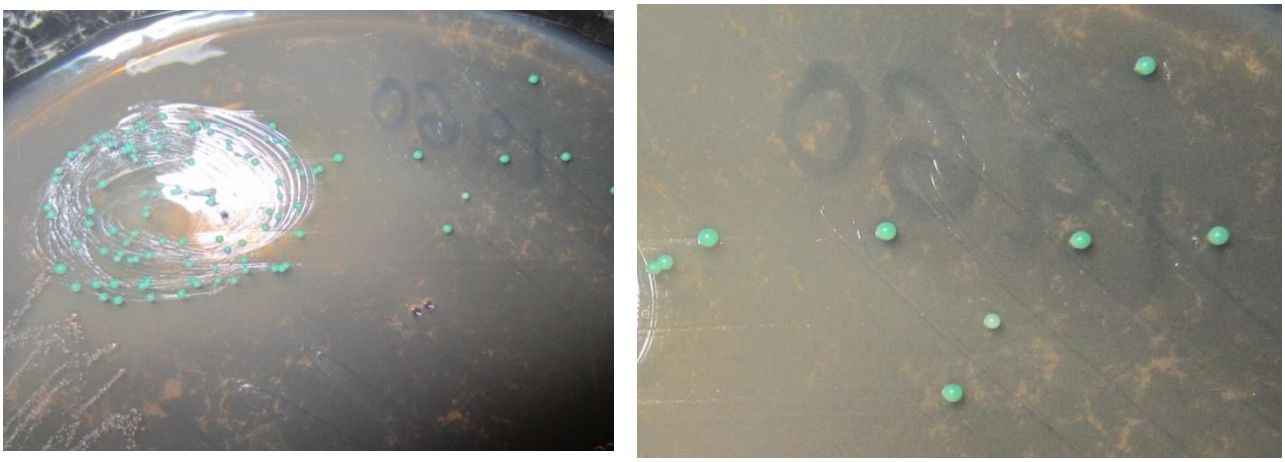


Figure 2: Candida albicans- Green colored colonies on Hicrome agar .

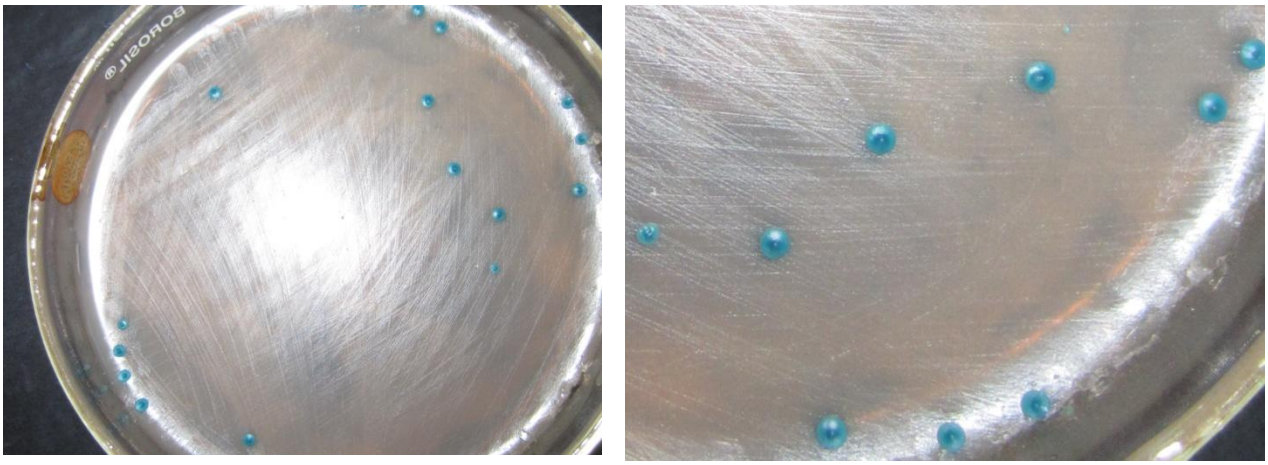


Figure 3: Candida tropicalis- Blue colored colonies on Hicrome agar

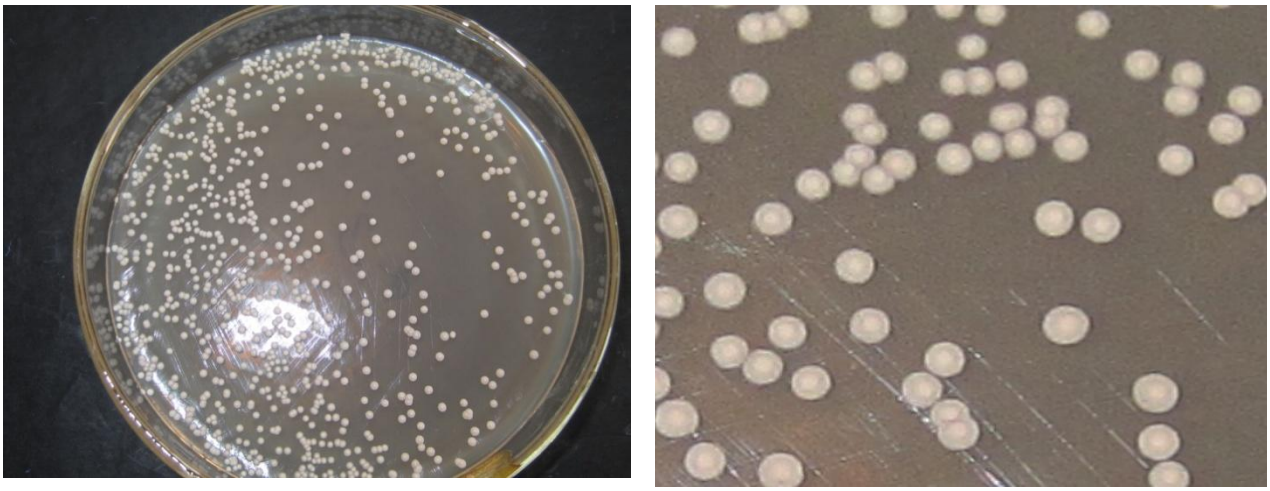


Figure 4: Candida parapsilosis- Pink colored colonies on Hicrome agar

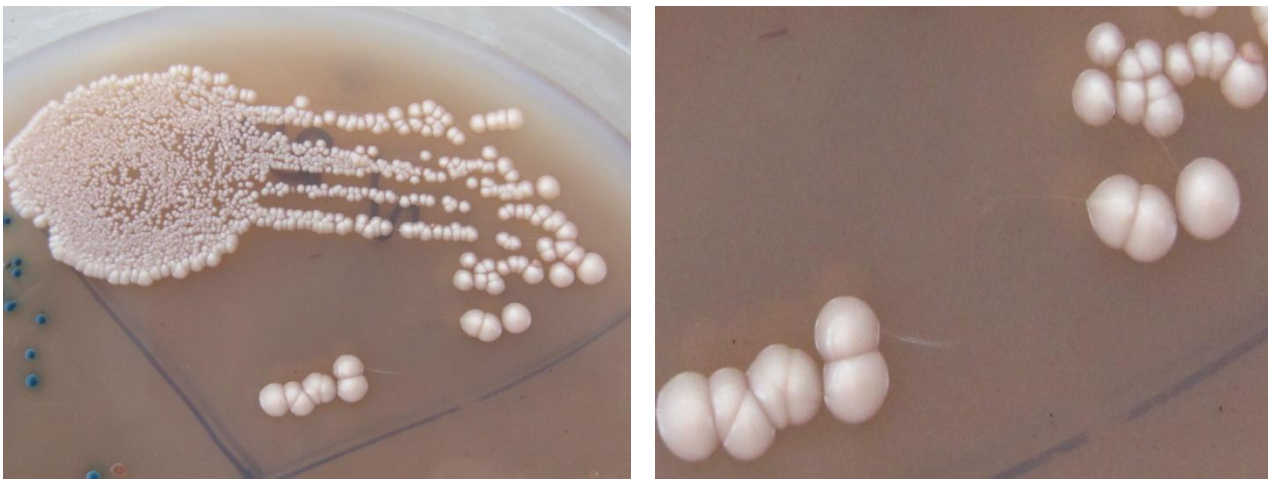


Figure 5: Candida kefyr- Cream- Pink colonies on Hicrome agar

Figure 6: Candida krusei- Pink colored colonies on Hicrome agar

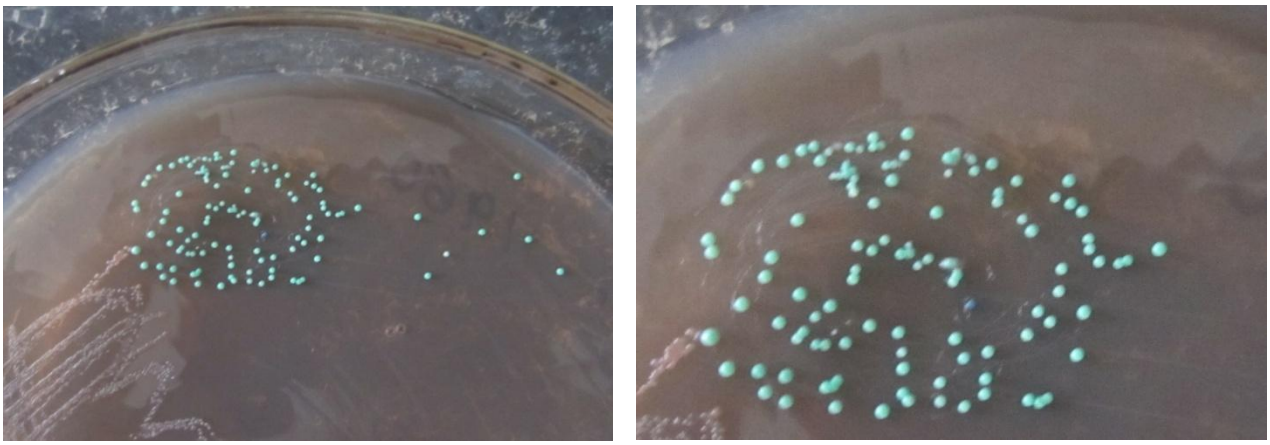


Figure 7: Candida guilliermondi- Greenish purple colonies on Hicrome agar