J. Egypt. Vet. Med. Ass. 52, No. 2, 121-129 (1992).

CULTURAL CHARACTERISTICS OF CANDIDA ALBICANS CRYPTOCOCCUS NEOFORMANS, RHODOTORULA RUBRA AND TRICHOSPORON CUTANEUM ON DIFFERANT BACTERIOLOGICAL MEDIA

M. REFAI*, F.R. EL-SEEDY**, MONA EL-ENBAAWY*, JAKEEN* EL-JAKEE and A.A. MOAWAD*

* Department of Microbiology, Faculty of Veterinary Medicine Cairo University.
** Department of Microbiology, Faculty of Veterinary Medicine Cairo University (Beni-Suef Branch).

SUMMARY: Four species of yeasts including *Candida albicans*, *Cryptococcus neoformans*, *Rhodotorula rubra* and *Trichospron cutaneum* were subcultured on different bacteriological media and incubated at 25°C and 37°C for 48 hours.All the inoculated plates and tubes were incubated under aerobic condition except the reinforced clostridial medium which was incubated under complete anaerobic condition, while brucella agar and thioglycolate media were kept under 10% Co_2. All the tested yeasts could not grow under complete anaerobic condition, but, *C. albicans* was the only yeast species that grew on brucella agar and thioglycolate media. The 4 species of yeasts could grow onto nutrient agar, blood agar, eosin methylene blue agar, dextrose tryptone agar, Lowenstein Jensen medium without sodium pyruvate, with glycerol as well as EMJH medium. *Rh. rubra* grew on all the used bacteriological media at 25°C but not at 37°C. On Simmon's citrate agar, mannitol salt agar none of

Received: 12.12.1991

the yeasts under test could grow. On MacConkey and violet red bil glucose agar both *Cr.neoformans* and *Tr. cutaneum* could tolerate the bile salts present in the both media and produced visible colonies. *Tr. cutaneum* was the only species of yeasts that could grow on Baird Parker medium with black colonies surrounded by opaque zone.

INTRODUCTION

Isolation and identification of viable pathogens are still the "gold standard" for diagnosis of infectious diseases today (Finegold and Baron, 1986). Mycological media differ from bacteriological media in a number of ways, because of the different requirements for growth. Most fungi have an optimum pH much lower than that of most bacteria, also fungi are more capable of growing on media of inorganic salts with the addition of carbohydrates as energy source,(Harrigon and McCance, 1976). Most fungi are strictly aerobic or facultative anaerobic microorganisms. Fungi can grow over very wide ranges of medium oxygen tension (Tabak and Cooke, 1968).

Frequently, yeasts develop nutrient requirements when they are grown at temperature approaching the upper cardinal point (Jones and Hough, 1970).

The present work was aimed therefore to find out how far yeasts can grow on various bacteriological media which are commonly used for the isolation of different bacteria and under the same conditions of incubation as they are commonly used for bacteriological work.

MATERIAL AND METHODS

1. Microorganisms:

Four species of yeasts, *C. albicans, Cr. neoformans, Rh. rubra* and *Tr. cutaneum* were isolated, from mastitic cows milk and faeces of diarrhoic calves, on Sabouraud dextrose agar with chloramphenicol and were identified according to Refai et al. (1969). Identification was through morphological characteristics on rice agar and by biochemical and physiological tests including, sugar fermentation, sugar and nitrate assimilation, urea hydrolysis as well as germ tube test using camel serum (Refai and El-Enbaawy, 1991) in addition to Indian ink preparation (Cruickshank et al. ,1975) for detection of the capsules.

2. Bacteriological media:

Different bacteriological media were prepared according to Oxoid (1982) including ordinary media e.g. nutrient agar, enriched media e.g. blood agar, differential media e.g. MacConkey agar and specific media for enterobacteriaceae e.g. eosin methylene blue agar, Simmons citrate agar and violet red bile glucose agar. Also dextrose tryptone agar for aerobic sporulation. Baird Parker and mannitol salt agar media for staphylococci, While for anaerobic cultivation, brucella agar, reinforced clostridial medium and thioglycollate agar were used. Lowenstein Jensen medium without sodium pyruvate, with glycerol (used for *Mycobacterium tuberclosis* and Ellinghausen medium modified by Johnson and Harris (EMJH) medium (used for leptospira) were also used.

C. albicans, Cr. neoformans, Rh. rubra and *Tr. cutaneum* were inoculated on the aformentioned bacteriological media. Each species of the four yeasts was cultivated onto 2 plates, one plate was inocubated at 37°C while the other one was at 25°C. All the media were

incubated under aerobic condition except reinforced clostridial medium which was incubated under complete anaerobic conditions using anaerobic jar. Brucella agar and thioglycolate agar plates were incubated under 10 % Co_2 produced by carbon dioxide generating kits. All cultures were examined after 48-72 hours and the results were recorded, the grown colonies were examined morphologically with regard to size, colour, surface, shape, edge and opacity.

RESULTS

C. albicans, Cr. neoformans. Rh. rubra and *Tr. cutaneum* could not grow on Simmon's citrate agar, mannitol salt agar (under aerobic conditions) and reinforced clostridial agar (under anaerobic condition). On the other hand, they could grow on nutrient agar, blood agar, eosin methylene blue agar and dextrose tryptone agar plates at both 25°C and 37°C exept *Rh. rubrua* which could grow at 25°C but not at 37°C. On blood agar, none of the tested yeasts produced haemolysis. On eosin methylene blue agar *Cr. neoformans* colonies were violet in colour while *Tr. cutaneum* colonies were pink. At 25°C *C. albicans* produced pin headed blue colonies.

On MacConkey agar, only *Cr. neoformans* and *Tr. cutaneum* could grow as non-lactose fermenter colonies. On violet red biole glucose agar, *Cr. neoformans* appeared as light violet colonies while *Tr. cutaneum* colonies were pink at 25°C and 37°C.

In the presence of 10 % Co_2 on brucella agar or in thioglycolate medium at 25°C or 37°C, *Cr. neoformans, Rh. rubra* and *Tr. cutaneum* had no growth after 48 hours incubation, but only *C. albicans* could grow as pin-headed, white, smooth, flat, entire and opaque colonies.

Tr. cutaneum was the only species which grew on Baird

Table 1: Growth of yeasts on different bacteriological media.

Media used	Candida albicans		Cryptococcus neoformans		Rhodotorula rubra		Trichosporon cutaneum	
	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
1. Nutrient agar	+	+	+	+	+	-	+	+
2. Blood agar	+	+	+	+	+	-	+	+
3. MacConkey agar	-	-	+	+	-	-	+	+
4. Eosin methylene blue agar	+	+	+	+	+	-	+	+
5. Simmons citrate agar	-	-	-	-	-	-	-	-
6. Violet red bile glucose agar	-	-	+	+	-	-	+	+
7. Dextrose tryptone agar	+	+	+	+	+	-	+	+
8. Barid Parker medium	-	-	-	-	-	-	+	+
9. Mannitol salt agar	-	-	-	-	-	-	-	-
10. Brucella agar	+	+	-	-	-	-	-	-
11. Reinforced clostridial medium	-	-	-	-	-	-	-	-
12. Thioglycollate agar	+	+	-	-	-	-	-	-
13. Lowenstein Jensen medium	+	+	+	+	+	+	+	+
14. EMJH medium	+	+	+	+	+	+	+	+

Parker medium after 48 hours incubation and the colonies were rough, opaque irregular at 37°C, changed into black coloured after further incubation, but at 25°C they were centrally black and surrounded by an opaque zone unbonated with entire border.

On Lowenstein Jensen medium, *C. albicans* and *Tr. cutaneum* had growth at 25°C and 37°C after 48 hours incubation *Cr. neoformans* had well growth at 25°C but weak growth at 37°C. *Rh. rubra* also had weak growth at 25°C but had no growth at 37°C.

Ellinghausen medium (EMJH) is a liquid medium in which all the tested yeasts could grow. Turbidity in the medium due to growth of *Rh.rubra* was moderate at 25°C and slight at 37°C while it was marked due to the growth of *Cr. neoformans* at 25°C and 37°C. Turbidity with foloculant growth appeared with *C. albicans* and clear solution with sediment appeared with *Tr. cutaneum* at 25°C and 37°C (Table 1).

DISCUSSION

C. albicans, Cr. neoformans, Rh. rubra and *Tr. cutaneum* could not grow on mannitol salt agar because they seem to be sensitive to high salts concentration in the medium. Also all of them could not grow on Simmon's citrate medium this is most probably due to the inability to utilize citrate as the sole source of carbon.

Smith (1961) employed reinforced clostridial medium with added blood and neomycin for determination of yeasts as well as other bacteria, while in our investigation under complete anaerobic condition none of the yeasts under test could grow in this medium. This is in agreement with John et al. (1975) who reported that obligate anaerobiosis in fungi had not been reported. All tested yeasts could grow on nutrient,blood

eosin methylene blue and dextrose tryptone agar media. Nutrient agar is a simple and unexpensive medium, while blood agar is enriched medium and is used also for the detection of haemolysis which is of diagnostic importance. The growth on blood agar without haemolysis indicated that *C. albicans*, *Cr. neoformans*, *Rh. rubra* and *Tr. cutaneum* had no haemolytic activities. Stockes (1976) reported that most fungi which cause human diseases well grow on blood agar incubated at 37°C but they grow more slowly than the majority of bacteria and it is necessary to incubate the cultures for 1 to 8 weeks, and to seal them so that they do not dry up. Some grow more quickly at room temperature. Walker and Huppert (1959) used eosin methylene blue agar for rapid identification of *C. albicans*, under 10 % Co_2 at 37°C and after 48 hours spidery or feathery colonies of *C. albicans* were developed, while other candida species produced smooth yeast like colonies. Also Finegold and Baron (1986) mentioned that, the appearance of spider like colonies on eosin methylene blue agar was characteristic of *C. albicans* On MacConkey medium and violet red bile glucose agar, only *Cr. neoformans* and *Tr. cutaneum* could grow. This means that they are bile tolerant, but *C. albicans* and *Rh. rubra* are bile sensitive. Baird Parker medium is a medium that contains lethium and potassium tellurite. It suppressed the growth of *C. albicans*, *Cr. neoformans* and *Rh. rubra* while allowing *Tr. cutaneum* to grow. Tellurite and egg yolk components are responsible for the formation of yellow colonies which changed with further incubation to black at 37°C, while at 25°C they were centrally black and surrounded by opaque zone, glycerinated Lowenstein Jensen medium contains malachite green (Difco, 1984). The rate of growth of the various species of yeasts on this medium indicated that *C. albicans* and *Tr. cutaneum* are not affected with malachite green, while *Cr. neoformans* and *Rh. rubra* are sensitive to malachite green in varying degrees depending on the incubation temperature. The turbidity in EMJH medium was characteristic for every species of yeast used

in the present investigation. All the aobve observation agree with Lennett et al. (1985) who stated that most yeasts were grew well on the common mycological and bacteriological media and produced visible colonies within 48-72 hours.

On the whole, the obtained results are of great practical importance. It is however, important to propogate the knowledge among the bacteriologist so as to recognize the yeast colonies on the various bacteriological media, so as not to miss such colonies. One point should be however considered, that is yeasts need at least 48 hours to grow, therefore bacteriological media should not be discarded before 2-3 days, better one week before they are considered negative for yeasts. They can first be examined after 18-24 hours for the rapidly growing bacteria, then reincubated and examined daily for yeasts.

REFERENCES

1. Cruickshank, R., Duguid, J.R., Marmion, B.P. and Swain R.H.A. (1975): Medical Microbiology. 12th Ed. Vol. II, Churchill Livingstone, Edinburgh, London and New York.

2. Difco Manual (1984): Dehydrated culture media and reagents for Microbiolog. Tenth Edition. Difco Laboratories, Detroit Michigan 48232, USA.

3. Finegold, S.M. and Baron, E.J. (1986): Diagnostic Microbiology. 17th Ed. C.V. Mosby Company, St. Louis, Toronto, Princeton.

4. Harringon, F.W. and Margaret, E., McCance (1976): Laboratory methods in food and dairy microbiology Acad. Press, Londin, New York, San Francisco.

5. John, E., Smith, D., David, R. and Berry, F.(1975): The filamentous fungi. Department of Applied microbiology, University of Strathcly de, Glasgow.

6. Jones, R.C. and Hough, J.S. (1970): The effect of temperature on metabolism of baker's yeast growing on continous culture. J. Gen. Microbiol., 60, 107-116.

7. Koneman, E.W., Allen, S.D., Dowell, V.R. and Sommers, H.M. (1979): Color Atlas and Textbook of Diagnostic Microbiology. J.B. Lippincott Company, Philadelphia, Toronto.

8. Lennette, E.H. Balowes, A., Husler, W.J. and Jean Shadomy, H. (1985): Manual of clinical microbiology Fourth Ed. American Society for Microbiology.

9. Oxoid Manual (1982): Culture media, ingredients and other laboratory services. 5th Ed. Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG 240 PW.

10. Refai, M., Gobba, A.H. and Rieth, H. (1969): Monograph on yeasts. Diagnosis diseases and treatment. Egypt. Vet. Med. J., 17, 255-316.

11. Refai, M. and Mona, El-Enbaawy (1991): Keimschlauchbioldung bei *Candida albicans* in Kamel serum. Hautnah Mykologie, 3, 166-167.

12. Smith W. (1961): J. Appl. Bact., 24 (3), 235-241. Cited by Oxoid Manual (1982).

13. Stockes, E.J. (1975): Clinical bacteriology.Fourth Edition. Edward Arnold Ltd. London.

14. Tabak, H.H. and Cooke, W.B. (1968): The effect of gaseous, environment on the growth and metabolism of fungi. The Botanical Review, 34, 126-252.

15. Walker, Leila, and Huppert, M. (1959): Am. J. Clin. Pathol. 31 (6), 551-558. Cited by Oxoid Manual(1982).