American-Eurasian Journal of Scientific Research 6 (3): 146-148, 2011
ISSN 1818-6785

Fishmeal Extract Dextrose Agar-A New Mycological Medium-A Preliminary Report

[1]K. Subbannayya [2] V. Arjuna Rao [3]P. Raghunath

[1]Department of Microbiology, KVG Medical College and Hospital, Sullia, 574 327
[2]Texas Women's University, Denton, Texas, USA
[3]Clinical Vaccine R & D Center, Chonnam National University Hwasun Hospital, 160 Ilsim-Ri, Hwasunup, Hwasun County, Jeonnam 519-809,Republic of Korea

Abstract: A new mycological medium, Fishmeal Extract Dextrose Agar (FEDA) was developed using aqueous extract of fishmeal as a substitute for peptone and evaluated for its efficacy in supporting the growth of fungi commonly encountered in clinical specimens and in maintaining fungal stock cultures. *Trichophyton rubrum, Trichophyton mentagrophyte*, *Microsporum gypsium*, *Microsporum nanum*, *Epidermophyton floccosum, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus*, *Mucor spp., Rhizopus spp., Cryptococcus neoformans* and *Candida albicans,* from stock cultures and/or from clinical specimens such as ear swab, urine and skin scrapings were successfully grown and preserved on FEDA containing 5% fishmeal extract, 2% dextrose and 2.5% agar at pH 7.0. The media was made selective for growing dermatophytes using antibiotics and cycloheximide. The results were compared with those on Sabouraud's dextrose agar (SDA) and found 100% conformity. An important advantage of FEDA was that dermatophytes were maintained better on this medium. Fishmeal, a cake obtained after extraction of oil from sardines used in the preparation of fishmeal extract was found to be 100 times cheaper, non-hygroscopic and could be stored without special precautions unlike peptone used in SDA. Hence FEDA may be a useful medium for primary isolation of fungi in culture and maintenance of stock cultures in the developing countries where stringent cost containment is a primary requirement in the laboratory diagnosis of diseases.

Key words: Fishmeal Extract Dextrose Agar · Fungi

INTRODUCTION

It would be beneficial if the same basal ingredient could be used in the formulation of several different culture media for growing microorganisms. Fishmeal extract may be one such ingredient which has already been successfully used in designing media in the *in vitro* cultivation of *Entamoeba histolytica,* isolation of medically important bacteria and their antibiotic sensitivity testing, prevention of swarming of Proteus and as a differential medium for lactose fermenting and non-lactose fermenting bacteria [1-5]. Present study described a fishmeal based culture medium, fishmeal extract dextrose agar (FEDA) developed for growing fungi.

MATERIALS AND METHODS

Fishmeal extract was prepared by boiling 5 g fishmeal (courtesy: Raj Fishmeal and Oil Company, Malpe, Udupi, India) with 100 ml de-ionized water for 10 minutes and filtering through Whatmann No. 1 filter paper. To this extract, 2 g dextrose and 2.5 g agar were added. Agar was steam dissolved, the pH of the medium was adjusted to 7.0 and the volume was made up to 100 ml. This medium, the Fishmeal Extract Dextrose Agar was steam sterilised at 115°C for 15 min. This medium is similar to Emmon's modified Sabouraud's dextrose agar (SDA) [6] in composition except that fishmeal was substituted for peptone in FEDA. The medium was made selective for growing dermatophytes by incorporation of antibiotics;

Corresponding Author: K. Subbannayya Department of Microbiology, KVG Medical College and Hospital, Sullia, 574 327, India.

streptomycin (40 units/ml), chloramphenicol (0.05 mg/ml) and cycloheximide (0.5mg/ml) as in SDA. Agar plates and slants were prepared with both kinds of FEDA.

Different filamentous fungi and yeasts were grown on plates and slants of FEDA and SDA using both standardized inocula of stock cultures (*Trichophyton rubrum, T. mentagrophyte*, *Microsporum gypsium*, *Microsporum nanum*, *Epidermophyton floccosum*, *Aspergillus niger*, *A. fumigatus*, *A. flavus, Mucor spp., Rhizopus spp., Cryptococcus neoformans* and *Candida albicans*) and from clinical specimens. Fifty specimens each of ear swab, urine and skin scrapings were also cultured on FEDA and SDA. Standardised inocula for both filamentous fungi and yeasts drawn from departmental stock cultures were separately prepared.

Inocula for Filamentous Fungi: Spore suspensions using adequate growth of moulds on SDA were prepared in 0.85% saline containing Tween 20 (1 drop to 1 ml of saline). After sedimentation of heavy elements, the suspension containing conidia/sporangiospores was adjusted to 10^4 CFU/ml.

Inocula for Yeasts: A 24h growth of yeasts was suspended in 0.85% saline and the turbidity of the suspension was adjusted to McFarland 0.5 standard (10^6CFU/ml).

Using a standard wire loop of 4 mm diameter, a wire loopful of the spore/yeast suspension was inoculated on plates and slants of both test and control media. For growing dermatophytes such as *Trichophyton mentagrophyte*, *Microsporum gypsium*, *Microsporum nanum* and *Epidermophyton floccosum* FEDA with added antibiotics and cycloheximide was used. Cultures were incubated at 28-30°C and observed for growth characteristics every day. Macroscopic appearance of the fungal colonies such as growth rate, shape, size, surface structure on the obverse, texture of colonies such as yeast-like, cottony, velvety, granular, consistency of the colony such as smooth or mucoid in case of yeasts, pigmentation on the obverse and reverse, diffusibility of pigments were observed on agar plates and slants. The results on FEDA and SDA were compared.

RESULTS AND DISCUSSION

All the fungi inoculated on FEDA grew and were maintained well. There was no difference in the growth rate, shape, size, surface appearance, texture, pigmentation, consistency of the fungal colonies, or microscopic appearance between fungi grown on FEDA and SDA. The efficacy of FEDA in the isolation of fungi from clinical specimens was comparable to SDA as shown in Table 1. However, stock cultures were maintained well for longer period on FEDA than on SDA.

Table 1: Comparison of FEDA with SDA in the isolation of fungi from clinical specimens

Clinical Specimen	Fungal Isolates	No. Isolates on FEDA	No. Isolates on SDA
Ear swab	*A. niger*	16	15
	A. fumigatus	10	10
	A. flavus	5	5
Urine	*C. albicans*	10	10
Skin scrapings	*T.rubrm*	5	4
	T. mentogrophyte	2	2
	E. floccosum	1	1

Whenever a growth medium is designed to grow particular microorganisms, it should have certain qualities. It should have ingredients that would support the optimum growth of the microorganism and express certain specific growth characteristics. The medium should be free from substances which are inhibitory to the microorganism. It should be cost effective and the ingredients of the medium are easily available.

Fishmeal is a dried powdered cake obtained after extraction of oil from sardines. It is found to be a good substitute for peptone [3]. Unlike peptone fishmeal is non-hygroscopic and does not become sticky on exposure to air. It can be stored at room temperature. Fishmeal has higher amino acid nitrogen content (2.62%) [3] than peptone (1.7%) [7] One of the important ingredients needed for the growth of fungi is NH4 form of nitrogen. Fishmeal also has phosphates, potassium, sodium, magnesium and low content of copper [5], the last being the desirable quality as copper salts is toxic to fungi. Fishmeal is also cost effective (approx. Rs.30/kg) than peptone (approx. Rs.3576/kg). The reduction in cost makes FEDA a good mycological medium for laboratories with limited financial resources. Fishmeal extract dextrose agar was found to be equal in efficiency to SDA in supporting the growth of various fungi, eliciting their macroscopic growth characteristics and in maintaining fungal stock cultures including moulds and yeasts. There was no difference in microscopic morphology of fungi grown on FEDA and SDA. Therefore, FEDA may be useful for primary isolation and maintenance of fungi in stock cultures in developing countries where stringent cost containment is a primary requirement in the laboratory diagnosis of diseases.

In conclusion this study has proved that FEDA is equal in efficacy to SDA in the isolation of fungi including pathogens such as dermatophytes from clinical specimens. The medium is cost effective and simple to prepare. Storage of fishmeal does not require any special precautions unlike peptone. Dermatophyte stock cultures can be maintained well on FEDA without losing their viability.

REFERENCES

1. Subbannayya, K., H. Babu, A. Kumar, KNA. Rao and PG. Shivananda, 1983. Fishmeal extract agar buffalo serum: A new diphasic culture medium for the diagnosis of amoebiasis – Preliminary studies. Indian J. Microbiol., 23: 126-127.
2. Mathai, A., K. Subbannayya and PG. Shivananda, 1985. Fishmeal extract agar- A new bacteriological medium-Preliminary report. Indian J. Pathol. Microbiol., 28: 392-332.
3. Subbannayya, K., P. Raghunath and V. Arjuna Rao, 2002. Fishmeal extract agar - a new antibiotic sensitivity test medium. Indian J. Exp. Biol., 40: 960-962.
4. Subbannayya, K. and J. Udayalaxmi, 2005. Fishmeal extract agar – a medium to inhibit swarming of *Proteus spp*. Curr. Sci., 89: 1666-1667.
5. Subbannayya, K., J. Udayalaxmi and M. Anugraha, 2006. Fishmeal extract bile salt agar – A differential medium for enteric bacteria. Indian J. Exp. Biol., 44: 675-678.
6. Emmons, CW., CH. Binford, JP. Utz and KJ. Kwon-Chung, 1977. Medical Mycology. 3rd edition, (Lea and Febiger), pp: 535.
7. Cruickshank, R., J.P. Duguid and RHA. Swain, 1972. Medical Microbiology. 11th edition, (English Language Book Society & Churchill Livingstone), pp: 737.