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Modified Fish Meal Extract Agar - A New Medium for the Selective Isolation of *Pseudomonas aeruginosa* - A Preliminary Report

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Abstract: A new bacteriological medium Modified Fish Meal Extract agar was tested for its efficacy in promoting the growth of *P aeruginosa* normally encountered in clinical specimens. 50 specimens each of sputum, pus and urine samples were cultured on Modified Fish Meal Extract Agar and the results were compared with normal Nutrient agar and Blood agar. There was 100% conformity in the results compared with the other selective media for Pseudomonas aeruginosa. One of the greatest advantages of this Modified FMEA was that no other organisms grew in culture other than *Pseudomonas aeruginosa*. The cost of consumable materials used in the preparation of fish meal extract agar being simple and cheap this media can be used successfully in the routine culture work for the isolation of *Pseudomonas aeruginosa* from clinical specimens like sputum.

Key words: Fish meal extract · *Pseudomonas aeruginosa*

INTRODUCTION

There has been continued effort in the recent years to improve the routinely used media in the bacteriological work. The aim has been to develop a simple and more economical medium for the isolation of Pseudomonas aeruginosa from clinical samples. Here we compare the efficacy of Modified Fish Meal Extract Agar with the conventional non selective and selective media for the effective isolation of *Pseudomonas aeruginosa*. Fish Meal Extract Agar (FMEA) has been already used in the cultivation of bacteria, Entamoeba histolytica, non swarming of the Proteus species, as Antibiotic sensitivity test medium and also as a Differential media for the lactose and non lactose fermenting organisms [1-5].

MATERIALS AND METHODS

Fish meal is ordinarily available in coastal Karnataka is nothing but the dried and powdered residue of sardine fish after the extraction of oil. This was the basic substance used in the preparation of modified fish meal extract agar.500 grams of Fish meal (Raj Fishmeal and Oil Co. Malpe) which was properly cleaned out of its gross impurities was boiled for 10 minutes in one liter of distilled water, with constant stirring. After cooling the solution was allowed to stand overnight and the supernatant was decanted this was the Fish meal extract concentrate [1].

For the preparation of modified fish meal extract agar for every 10 ml of fish meal extract 90 ml of distilled water was added making the final concentration of fish meal in the medium to 5% beef extract 1% sodium chloride and dettol 0.3% was added to make the medium selective. The pH of the medium was adjusted to 7.4 and 2% of the agar was added. The medium was sterilized by autoclaving at 121°C and poured into Petri plates [6].

50 specimens of each of sputum urine and pus were cultured on Modified Fishmeal Extract Agar, Blood agar, Nutrient agar, Cetrimide agar and Pseudomonas isolation agar and the results were compared.

OBSERVATIONS AND DISCUSSION

Pseudomonas aeruginosa grew on all the plates and there was cent percent conformity in the isolation of this organism from the sputum, urine and wound specimens. This study was also used to compare this medium with the Pseudomonas isolation agar and cetrimide agar it was noted that the isolation of pseudomonas aeruginosa when less in sputum samples were better in the Modified Fish

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Table 1: Comparison of different culture Media in isolation of *Pseudomonas aeruginosa* from different clinical specimens

Specimens (50 samples each)	Blood agar*	Nutrient agar*	Cetrimide Agar**	Pseudomonas isolation agar**	Modified fishmeal extract agar**
Pus	12	12	12	12	12
Sputum	17	17	15	17	17
Urine	5	5	5	5	5

*In these media along with the *Pseudomonas aeruginosa* other bacterial were also grown

**These were the selective media used for comparing the selective isolation *Pseudomonas aeruginosa*

Meal Extract agar as shown in Table 1 compared to the Cetrimide media but the intensity of pigment production was found to be less in the Modified Fish Meal Extract Agar compared to the above two selective media. The two strains of *Pseudomonas aeruginosa* isolated from sputum produced pyorubin pigment in the Modified Fish meal Extract agar. The colonies were more mucoid in nature after 24 h of incubation Modified Fish meal extract agar where as the colonies were highly pigmented and irregular in nature on cetrimide and Pseudomonas isolation agar.

The Fish meal extract agar without any added ingredients were also tried in this study but none of the medically important bacteria grew in the medium in 24 h. Only *Pseudomonas aeruginosa* grew in the culture after 48 h of incubation the colonies were very small and highly irregular in morphology. To isolate the organism in a faster rate the fish meal extract medium was modified which had similar isolation rates at par to the common selective medias used for the isolation of *Pseudomonas aeruginosa*.

The cost of the consumable materials used in the preparation of 100 ml medium is very much lower than that of other selective media. Hence this is a much cheaper medium than Pseudomonas Isolation Agar and Cetrimide agars which are used for the selective isolation of Pseudomonas from clinical specimens and this could be successfully replaced with the former in routine bacteriological work.

Nutritionally fish meal extract is as good as peptone and has been successfully used for formulating media to grow bacteria and to test their antibiotic susceptibility. The performance of the media with regard to growth characteristics were largely at par with each other. However bacteria isolated from clinical specimens produced large colonies than those used from stock cultures. The counts of growth on all these media were same. The growth characters of the *Pseudomonas aeruginosa* isolated from clinical specimen is more important than those sub cultured from stock cultures. Upon storage stock cultures usually tend to lose some of their growth characteristics.

Unlike peptone fish meal is non hygroscopic and do not become sticky when exposed to air. Like peptone fish meal has a very low content of copper and is free from fermentable carbohydrates and able to support the growth of moderately exacting bacteria like S aureus. In addition fish meal has higher amino acid nitrogen 2.62% and tryptophan 1.87% than peptone for which the values are 1.7 and 1.2% respectively [5]. The cost of fish meal is much low Rs 16/kg than peptone 1600/kg. In addition to the quality of growth in microbiology the important aspect is to obtain the raw materials very cheaply for routine clinical investigations. Hence modified Fish meal extract agar may be a suitable alternative compared to other selective media in the primary isolation of *Pseudomonas aeruginosa* from clinical samples like sputum which is often contaminated with normal oral flora which was observed on blood and nutrient agar.

According to the previous studies it is observed that none of the selective media produce the growth of pseudomonas species in the primary isolation and it is better to inoculate the specimen in a noninhibitory medium and then subculture on to the selective media for the proper isolation of Pseudomonas species [7]. In this study we found that the Modified Fishmeal Extract Agar media is far superior to other selective media which are commonly used for the isolation of *Pseudomonas aeruginosa* from clinical specimens as even a very scanty growth of *Pseudomonas aeruginosa* could be observed in the Modified Fishmeal extract agar for the selective isolation of *Pseudomonas aeruginosa*.

In conclusion this study has proved that in the selective isolation of *Pseudomonas aeruginosa* from clinical specimens the modified Fish meal extract was better in the isolation rate compared to the Pseudomonas Isolation Agar and Cetrimide agar. This media is also found to be very cost effective as the raw materials used is very cheap compared to the other selective media hence this media can be used for the selective isolation of *Pseudomonas aeruginosa* which is obtained as a pure growth on the agar and is within the reach of every microbiology laboratory for routine bacteriological investigations whenever pseudomonas infections are suspected.

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