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Comparative Evaluation of EMB agar and chromogenic Coliform Agar for detection of *E.coli* in water samples.

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Abstract

Fresh water is essential for life. Water sources like icebergs, bogs, ponds, lakes, rivers, streams percolate underground forming ground water. There are chances that the fresh water sources may get contaminated with faecal matter. *Escherichia coli* is the most common member of faecal coliforms and an indicator of faecal water contamination.

In this study freshwater samples were collected from Mangalore and analysed for the coliform and mainly*E. coli*. Dissolved oxygen (DO) content of water was also calculated.The freshwater samples were seeded inEosin Methylene Blue (EMB) and Hicrome Coliform Agar (HCA)to differentiate *E.coli* from others coliforms. *E.coli* on EMB gives metallic sheen colonies and blue coloured colonies on HCA.Out of the HCA and EMB Agar, HCAshowed greater number of, *E.coli* colonies alongwith other coliforms.

Lowered DO in the water indicates high amount of microbial activity degrading organic matter. Thus water samples with higher colony count on HCA correlated with the low DO content.Hence the HiCrome Coliform Agar can be used for the selective,rapid and qualitative identification of *E. coli* from the water samples.

Key words: Potable water, *E. coli, Hi* Coliform Agar,EMB,Dissolved oxygen

Introduction:

Clean and pure drinking water is the necessity of every living being. Though earth is covered by 70% of water, only 3% of it is freshwater. Around 60 percent of the human body is made up of water and on an average 3lit of water is consumed by anadult

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human.(1)Freshwater is used for drinking, to irrigate crops, in industries, factories and as part of sanitation system.

Availability of fresh and clean water is a requirement in health and food production (2).There are chances that the fresh water sources may get contaminated with faecal matter.*E.coli* are found to be in elevated densities in faecal matter,sewage and water contaminated with faecal matter. Hence it is considered as the faecal indicator microorganisms(3, 4). *E.coli* is a Gram negative bacteria found commonly in lower intestine of warm blooded mammals (5).It can get into water by rain, snow and it may enter into rivers, streams ,lakes or groundwater from land surface(6,7).Most *E.coli* strains are harmless but serotype 0157:H7 can cause serious food poisoning in humans (8,9).Levels of *E.coli* cannot exceed 575 CFU per 100ml of water for partial body contact(10). As per the drinking water standards, water should be free from any microbes.*E.coli* may be present at low levels in water in every season.

To grade the drinking water quality, the detection of *E.coli* in water sample is crucial to rule out the sewage contamination.In the Most probable number test of water quality, *E.coli* growing with metallic sheen on Eosin Methylene blue agar is the final confirmatory step to indicate the faecal contamination of water. The chromomeric substrate can be used to selectively detect the pathogens, coliforms and *E.coli* in surface water at a faster rate (11, 12).Hi Crome Coliform Agar (HAC) is a differential medium used here for the detection of *E. Coli* and other coliforms from water samples. Each coliform gives different coloured colony on HAC thereby making easy identification.

Hence the current study compares chromogenic media Hi Chrome Coliform Agar (HCA) and Eosin ethylene blue agar (EMB) for detectionof *E.coli* from freshwater samples.

Dissolved oxygen (DO) level of water indicates about the extent of contamination of water bodies by organic matter. Hence the dissolved oxygen of water samples were also analysed and correlated with the bacterial growth on respective media.

Methodology

1. Sample collection

The water samples were collected in sterile 500ml wide mouth plastic bottles kept on ice for transportation and processed within 24 hours.Totally 15 samples were analysed comprising 5 samples from river, well and pond water.

2. Determination of dissolved oxygen

The level of dissolved oxygen in water determines the water quality because it indirectly indicates the level of pollution. Dissolved oxygen measurements calculate the amount of gaseous oxygen dissolved in surface water. The amount of dissolved oxygen in freshwater samples was estimated by Azide Winkler method (13). The potable water should have the DO concentration of 6.5-8mg/L

3. Growth of *E. coli* on EMB Agar and HiCrome Coliform Agar

The purpose of this investigation was to compare EMB agar M317 (HiMedia) with HiCrome Coliform Agar modified M1832 (HiMedia) for thedetection of *E. coli*.1ml of different freshwater samples of river, Wells

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and ponds were mixed with respective media and poured into Petri plates. Plates were incubated for 24 hours at 37°Cwere observed for development of colonies.

4. Biochemical characterization of selected colonies.

Six selected colonies were selected and subjected to biochemical tests. These tests were done using KB003 Hi25 Enterobacteriaceae identification kit which is a standardized colorimetric identification system utilizing 13 conventional biochemical tests and eleven carbohydrate utilization tests. The test is based on substrate utilization and pH change. The pure cultures were inoculated into strips and incubated for 24 hours at 37°C and results were tabulated.

Results and Discussion

Dissolved oxygen content of Fresh Water sample

Dissolved oxygen measures the amount of gaseous oxygen dissolved in aqueous solution. Dissolved oxygen concentration is an important indicator of biological activity of water.Healthy water should generally have dissolved oxygen concentrations above 6.5-8 mg/L.(14) Dissolved oxygen content of second and fifth Pond water had the lowest dissolved oxygen concentration i.e. 2.8mg/l. and 4.5mg/L(Table1) respectively.

When there is an organic discharge dissolved oxygen decreases rapidly due to the action of the aerobic microorganisms that consume oxygen during metabolic degradation of organic matter. Dissolved oxygen level of 6 mg per litre is sufficient for most aquatic species. DO levels below 4 mg per litre are stressful to most aquatic animals and DO below 2 mg per litre will not support aerobic aquatic life Environmental input of total dissolved solids concentration in drinking water should not exceed about 13 to 14 mg per litre (15).

Table 1: Comparative Evaluation of EMB and HiCrome Coliform Agar for the detection of *E.coli*.

Water sample	Dissolved oxygen mg/L	Colonies of *E. coli*	
		HiCrome Coliform Agar (Blue colonies)	EMB Agar(Green metallic Sheen colonies)
P1	5.3	5	2
P2	2.8	98	50
P3	6.9	4	2
P4	7.8	Nil	1
P5	4.5	78	20
W1	6.9	Nil	Nil
W2	6.6	4	Nil
W3	7.2	Nil	Nil

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W4	7.4	Nil	Nil
W5	6.9	5	1
R1	7.8	1	1
R2	8.6	3	1
R3	8.7	15	8
R4	5.7	17	10
R5	7.7	5	2

Note- P-Pond, W-well, R-River water samples

Evaluation of *E.coli* growth EMB and HiCrome Coliform Agar

On Hicrome Coliform Agar a greater number of blue colour colonies were observed than number of colonies with metallic sheen on EMB.*E. coli* forms dark blue colonies on Hicrome coliform Agardue to breakdown of chromogens (16, 17, and 18). Other water samples also showed he Coliform growth but second and fifth Pond water sample showed a greater number of *E. coli* colonies on Coliform Agar media.

The HiCrome Coliform Agar was found to be most efficient media for detection of *E. coli*. In Hi Chrome Coliform Agar, peptone and yeast extract provide essential growth nutrients to the organisms. The colour develops due to magnesium sulphate present in medium. Chromogenic mixture contains two chromogenic substrate which enables the detection of two specific enzymes beta galactosidase and beta glucoronidase. Beta galactosidase produced by coliforms cleaves one chromogen resulting in pink colour colonies of coliforms.But the enzyme beta glucouronidase produced by *E.coli* cleaves X- glucouronide. Thus *E.coli* forms dark blue colonies due to breakdown of both chromogens .Other Gram negative bacteria produced white and colourless colonies.On EMB agar, *E.coli*was identified based on the occurrence of green metallic Sheen (19).

For biochemical test, on incubation, organisms undergo metabolic changes which were indicated by a colour change in the media that is either visible spontaneously or after addition of a reagent. The blue coloured colonies were confirmed as *E.coli*, White or colourless colonies were of Enterobacter and pink coloured colonies were confirmed asKlebsiella.

Conclusion

In this study,two pond water samples had high *E. coli* and coliform count on Hicrome Coliform Agar. The same samples showed low dissolved oxygen concentration.This shows the extent of water pollution with organic matter and increased microbial activity.

The colored colonies of *E. coli* and other coliform on HAC and EMB agar correlated with the Enterobacteriaceae identification kit results. Comparative evaluation of EMB and Hicrome Coliform Agar showed there were a greater number of *E.coli* colonies on HiCrome coliform agar than onEMB.Thus HiCrome Coliform Agar seems more

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efficient media for the selective and rapid identification of *E.coli* from water samples.

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