Iranian J. Publ. Health, 1998, Vol. 27, Nos. 3 - 4

DEVELOPMENT OF NEW MEDIUM TERMED ECOL FOR THE DETECTION OF *ESCHERICHIA COLI*

M.K. Sharifi-Yazdi [1] , MSc, PhD ; C. Azimi [1] , DMD, PhD

Key words : *Indicator bacteria, faecal coliform, E. coli*

Abstract

A new medium, termed Ecol, was developed for the detection and enumeration of *Escherichia.coli.*

The selective properties of Ecol medium for the detection of *E.coli* form mixed culture in wastewater was tested and compared with conventional coliform media at 37°C and 44°C. Results clearly showed that ECOL medium was superior to the other tested media for the selective enrichment of *E.coli.*

The addition of L-tryptophan to the ECOL medium was tested to enable direct confirmation of *E.coli* to be carried out by the indole reaction. The results indicated that this was an excellent single step medium for *E.coli* enumeration by the most probable number procedure.

Introduction

The term indicator organisms as used in water microbiology means a microorganism whose presence is evidence that pollution has occurred. The most widely used indicator of sanitary quality of water is the coliform group of bacteria and *E.coli* in particular. *E.coli* is the essential of pollution by faecal material of human or animal origin.

The term *E.coli* refers to thermotolerant coliform organisms which ferment lactose (or manitol) at 44°C with the producation of acid and gas within 24 hours (14).

The coliform organisms and *E.coli* usually estimated by either the most probable number (MPN) method or the membrane filtration (MF) technique (14).

The variety of media has been developed for testing coliform and *E.coli.*

1- Dept. of Medical Laboratory Sciences, Faculty of Paramedicine, Tehran University of Medical Sciences, P.O.Box 14155-6446 , Tehran, Iran.

these media were selected either by use of inhibitory substance which suppresses the growth of non-coliform organisms, such as bile salts or choosing chemically defined nutrients which can be utilized by limited number of bacteria (1,5,10, 11, 12,13).

Isolation and enumeration methods for indicators in dirnking water invariably rely on the use of selective culture media. The limitations of selective media are, that most selective media are either insufficiently selective or inhibitory for the group of organisms they are supposed to enumerate (8,2).

Until now *E.coli* is still the most reliable indicator for potable water. The aim of this research was to develop a medium, in order to minimize the limitations of conventional media.

Materials and methods

The conventional media used, were Mineral Modified Glutamate Medium (MMGM), and Lauryl Tryptose Broth (LTB). Both of them were recommended media, Report 71(14) as presumptive and confirmatory media for the detection of *E.coli* in water.

10 ml of medium was dispensed into each of 360 screw-capped (1-oz) bottles, containing an inverted Durham tube, after autoclaving 1 ml of wastewater, sample was inoculated into each bottle of the 120 inoculated bottles of each medium, 60 were incubated at 37°C in an incubator and 60 were incubated at 44°C in a thermostatically controlled water bath for 24 hours.

The bottles were examined for gas and acid production for 24 hours. Then a loopful form each bottle was streaked an eosin methylene blue agar (EMB) plates. Such plates were incubated at 37°C for 24 hours.

The plates were examined at 24 hours for the presence or absence of green metallic sheen colony (when present or non-metallic sheen when absent) form EMB plates was picked and re-streaked on a fresh EMB agar plate. The plates were incubated at 37°C for 24 hours. This procedure was repeated up to four times to make sure that isolates were pure cultures.

All the isolated cultures were transferred on to nutrient agar slopes in 1-oz bottles and were incubated at 37°C for 24 hours.

The nutrient agar slopes containing pure cultures of isolates were labelled and stored at 4°C until needed.

Prior to the start of identification, the isolates were plated out on EMB agar to make sure they were not contaminated. The Gram-reaction was determined and colonial appearance on EMB agar was recorded.

The API system used were API 20 E, which is designed for the identification of Enterobacteriaceae.

The API system was used according to the procedures provided by the API manufacturer. Possible identification of isolates were made with the aid of the API differential chart and the analytical profile recognition system.

For the possible suitability of ECOL + Tryptophan to be used as a single confirmatory medium for the detection of *E.coli* by MPN method, first the type and quantity of tryptophan was determined, by the separate addition of L, D and DL tryptophan into ECOL medium. 10 ml of medium was dispensed into test tubes cotaining an inverted Durham tube. A loopful of *E.coli* was inoculated into each test tube and incubated at 37°C for 18 hours. A total of 290 *E.coli* strains, previously isolated from wastewater samples, were used. The *Enterobacter aerogenes* was used as negative control after 10 hours incubation gas production was detected. A few drops of Kovac's reagent were then added into each tube. The tube was gently shaken and the development of a deep red colour in the upper layer was taken as a positive indole test. The experiment were repeated at 44°C with the same procedures.

Results

The following formula is the ingredients of ECOL medium, which was found to be the most suitable for the detection of *E.coli*, in terms of positive growth, optimal inhibitory level for the selectivity properties, and occurrence of false-positive and false-negative results.

ECOL medium

Ingredients	Gram/Litre
Proteose peptone	20
Lactose	5
Sodium chloride	5
Sodium dihydrogen phosphate	1
Sodium lauryl sulphate	0.3
Bile salts	1
Bromcresol purple	0.02

Tables (1,2,3) shows the API results of cultures originally isolated on MMGM, LTB and ECOL media at 37°C and 44°C. Of 60 isolates at 37°C on MMGM, 41 (68.3%) were identified as *E.coli* and of 60 isolates at 44°C, 53 (88.3%) were identified as *E.coli*. Of 60 isolates at 37°C on LTB, 38 (63.3%) were identified as *E.coli* and of 60 isolates at 44°C, 55(91.6%) were identified as *E.coli*. Of 60 isolates at 37°C on ECOL medium, 46(76.6%) were identified as *E.coli*, and of 60 isolates at 44°C , 59 (98.3%) were identified as *E.coli*.

The idea of using ECOL medium as a single confirmatory medium for the detection of *E.coli* by addition of tryptophan into medium was considered (10). It was found that addition of 0.02% tryptophan and autoclaving at 115°C for 10 minutes was suitable for the indole test. Positive indole test was obtained with all the 190, *E.coli* strains in (Ecol+0.02% tryptophan) both at 37°C and 44°C. In the earlier tests for identification, by the API, all of these *E.coli* strains were positive in the indole test at 37°C.

Discussion

The use of coliforms and faecal coliform bacteria as indicators of water quality has led to the development of new media and techniques. Selective media are designed to allow the growth of specific organisms or groups of organisms and to prevent the growth of all others (2,3,4,6,7,9).

In this study a quite large number of cultures isolated at 37°C incubation temperature in MMGM and LTB showed abundant mucoid colonies on EMB plates with occasional green metallic sheen colonies. This probably reflects the growth of a large number of non-*E.coli* organisms, and the presence of capsule-producing organisms, such as klebsiella species. The copious quantities of capsular material produced by these organisms can have interferance with the isolation of *E.coli* on EMB plates.

The ECOL medium was optimized in terms of components and concentrations for the detection of *E.coli* at 44°C. The medium performed well for the selective enrichment of *E.coli* from wastewater. Also it was more selective for *E.coli* than conventional MPN media used in water testing.

The L-tryptophan was added into ECOL medium for indole production by *E.coli*. The positive gas and indole test results with 190 strains in (Ecol+0.02% L-tryptophan) in the test tube at 44°C, showed that it may well be possible to utilize the medium as a single stage MPN enumeration medium which would allow both the enrichment and confirmation of *E.coli* cells in the same tube. Conventional procedures required a two stage enrichment and confirmatory procedure.

Table 1- The characteristics and API results of strains from wastewater isolated in MMGM at 37°C and 44°C.

Isolation temp.	No. of isolates	Morphology	Gram-reaction	GMS	N-GMS	API Results		
						No. of *E.coli*	No. of unidentifable	No. of other isolates indentified
37°C	60	All rods	All negative	45	15	41	15	1 *Serratia marcescens* 1 *Klebsiella pneumonia* 1 *Enterobacter cloacae* 1 *Salmonella spp*
44°C	60	All rods	All negative	55	5	53	7	0

GMS : Green metallic sheen colonies
NGMS: non-green metallic sheen colonies

Table 3- The characteristics and API results of strains from wastewater isolated in ECOL medium at 37°C and 44°C.

Isolation temp.	No. of isolates	Morphology	Gram-reaction	GMS	N-GMS	API Results		
						No. of *E.coli*	No. of unidentifable	No. of other isolates indentified
37°C	60	All rods	All negative	51	9	46	5	3 *Enterobacter agglomerans* 2 *Enterobacter cloacae* 1 *Klebsiella pneumoniae* 1 *Shigella* 1 *Yersinia* spp 1 *Pseudomonas* spp
44°C	60	All rods	All negative	60	0	59	1	0

GMS : Green metallic sheen colonies
NGMS: non-green metallic sheen colonies

Table 2- The characteristics and API results of strains from wastewater isolated in LTB medium at 37°C and 44°C.

Isolation temp.	No. of isolates	Morphology	Gram-reaction	GMS	N-GMS	API Results		
						No. of *E.coli*	No. of unidentifable	No. of other isolates indentified
37°C	60	All rods	All negative	41	19	38	17	1 *citrobacter freundii* 1 *citrobacter spp* 1 *Enterobacter agglomerans* 1 *serratia liquefaciens* 1 *pseudomonas spp*
44°C	60	All rods	All negative	56	4	55	5	0

GMS : Green metallic sheen colonies
NGMS: non-green metallic sheen colonies

1- Ciebin, B.W. (1995): Comparison evaluation of meodified m-FC and m-TEC media for membrane filter enumeration of *Escherichia coli* in water. Appl. Enviro. Microbiol, Nov. **61**(11): 3940-2.

2- Gray, R.D. (1964): An improved formate lactose glutamate medium for the detection of *Escherichia coli* and other coliform organisms in water. Journal of Hygiene. Cambridge **62**: 495-508.

3- Hajna , A.A. and Perry , C.A. (1943) : Comparative study of presumptive and confirmative media for bacteria of the coliform group and for faecal streptococci. American Journal of Public Health. **33**: 554-560.

4- Howard, N.J. ; Lochhead, A.G. ; McGrady, M.H. (1941): A study method for the detection of the presence of coliform organisms in water. Canadian Journal for Public Health. **32:** 29-36.

5- Kalthenthaler , E.C. ; Drasar , B.S. ; Potter , C.W. (1996): The use of Microbiology in the study of hygiene behaviour. Microbios. **88**(345): 35-43.

6- Lee , R.J. (1991): The microbiology of drinking water. Medical Laboratory Science. **48:** 303-313.

7- MacConkey , A.T. (1908) : Bile salts media and their advantages in some bacteriological examination. Journal of Hygiene. **28:** 322-323.

8- Mallman , W.L. and Darby, C.W. (1941): Uses of a Louryl Sulphate Tryptose Broth for the detection of coliforms. American Journal of Public Health. **31:** 127-134.

9- Mc Grady, M.H. (1937): A practical study of procedures for the detection of the presence of coliform organisms in water. American Journal of Public Health. **27:** 1243-1253.

10- Munoz, E.F. and Silverman, M.P. (1979) : Rapid, Single step Most-Probable Number method for enumerating faecal coliforms in effluents from sewage treatment plants. Journal of Applied and Environmental Microbiology. **37:** 527-530.

11- PHLS (1969): A mineral modified glutamute Medium for the enumeration of coliform organisms in water , by Public Health Laboratory Service Standing Committee on the bacteriological examination of water supplies. Journal of Hygiene. **67:** 367-374.

12- PHLS (1980): A comparison between Mineral Modified Glutamate Medium and Lauryl Tryptose Lactose Broth for the enumeration of *Esherichia coli* and coliform organisms in water by the Multiple Tube Method, by a Joint Committee of the Public Health Laboratory service and the Standing Committee of Analysts. Jouranl of Hygiene. **85:** 35-49.

13- PHLS (1981): A comparison of confirmatory media for coliform organisms and *Escherichia coli* in water , by a Joint Committee of the Public Health Laboratory Service and the Standing Committee of Analysts. Journal of Hygiene. **87:** 369-375.

14- Report 71 (1985): The Bacteriological Examination of water supplies, HMSO, London.