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Flourogenic Assays for Rapid Detection of *Escherichia coli* in Tap Water and Raw Milk Samples

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Abstract: As rapid microbiological reports of foods and food related products for *E. coli* bacteria presence are of prime importance. Rapid assays for *E.coli* were developed by using the compound 4-methyleumbelliferyl glucuronide (MUG), which is hydrolyzed by glucuronidase (GUD) to yield a fluorogenic product. The production of glucuronidase was limited to strains of *E.coli* in the family of *Enterobacteriaceae*. For rapid confirmation of the presence of *E.coli* in most probable number tubes (MPN), MUG was incorporated into lauryl tryptose broth at a final concentration of 100 μg ml^{-1}. Results of both the presumptive test (gas formation) and the confirmed test (fluorescence) for *E.coli* were obtained from tap water and raw milk samples after incubation of 6-24 hours at 45^0C, Approximately 94% of the tubes showing both gas production and fluorescence contained fecal coliforms (they were positive in EC broth incubated at 45^0C). Few false positive reactions were observed. The lauryl tryptose broth MUG most probable numbers assay was superior to conventional study for *E. coli* detection. The fluorogenic assay was sensitive and rapid but conventional study was time consuming and laborious.

Key words: Fluorescence, MUG, GUD and *E. coli*

Introduction

Coliform Fecal coliform or *E. coli* are considered the most important and compulsory measure of microbiological quality of food and food related products in terms of hygiene. Coliform, Fecal coliform or *E.coli* are all used as indicators of fecal pollution. Among these, *E.coli* is often preferred as an indicator because it is specific and most reliably reflects fecal origin. Conventional tests for coliforms and fecal coliforms or *E.coli* are time consuming in which require 5-6 days and deficiency in both precision and specificity. Therefore, has been placed on developing rapid tests for identifying *E.coli* as an indicator of direct or indirect fecal contamination in foods and food related products, The developments of rapid methods which are highly specific for identifying *E. coli* have been reviewed (Hofstra and huis in 't veld 1988; Hartman, 1989). In this review, stress has been placed on the usefulness of a variety of methods used in *E. coli* assays based on detecting β-glucuronidase (GUD) activity. Although the bacterial enzyme was discover first in *E.coli* (Buchler *et al.,* 1951). Its relative specificity for identifying this organism was not apparent until Kilian and Bulow (1976, 1979) surveyed the *Enterobacteriaceae* and the vibrionaceae and reported that GUD activity was mostly specific to *E.coli*.

In addition, the MPN assay is susceptible to bacterial interference (Allen *et al*., 1975; Geldreich *et al*., 1972; Anderson *et al.,* 1980) false negative reactions (absence of gas production in the presence of coliforms) may occur at the presumptive, confirmed and completed steps of the MPN analysis (Evans and LeChevallier, 1981). Other factors, such as synergistic gas production from lactose by non-coliforms (Greer and Nyhan, 1928), cultivation of an aerogenic and non lactose fermenting *E.coli* strains (Anderson, Meadows, Mullins and Patel, 1980) and the presence of lactose fermenting non coliform (Hussong *et al*., 1980; Hussong *et al.,* 1981), have contributed to the inefficiency of *E.coli* detection methodologies. The use of microbial enzyme profiles to detect indicator bacteria is an attractive alternative as existing method. Enzymatic reactions are specific, rapid and sensitive. For example, approximately 97% of the *E.coli* strain examined by (Kilian and Bulow, 1976) produced β-glucuronidase almost all other Enterobacteriaceae organisms lacked the enzyme GUD activities were initially detected with chromomeric substrates (Fishman, W.H., B. Springer, 1948); however a more sensitive assay with the fluorogenic compound 4-methyl umbelliferryl glucuronide has been used in recent years (Dahlen and Dahlen, 1973; Maddocks *et al*., 1975; Mead *et al*., 1955). GUD cleaves MUG to release a

fluorogenic end product that is visible under long wave (366 nm) UV light. The present study was under taken to developed rapid, specific and more efficient detection assays for *E.coli* by using the fluorogenic substrate MUG to detect GUD positive bacteria.

Materials and Methods

Specificity studies: The presence of GUD was determined by using a medium developed by Dahlen and Linde, 1973. To assay for GUD activity, growth from overnight cultures was stabbed into individual wells. The plate was incubated overnight at 37^0C and examined for the appearance of fluorescence under long wave UV-light (Black light blue, Westinghouse [Bloomfield, N.J]; emission about 366nm) Because of their short wavelength, most germicidal lamps are not suitable for this purpose.

Application of MUG: The possibilities of using MUG directly in conventional coliform media were examined by using coliform detection methods. All media were prepared as specified by the manufactures. In the MPN method, MUG was incorporated into the presumptive medium. Appropriate concentrations of MUG were dissolved in distilled water and mixed with lauryl tryptose broth to obtain a final substrate concentration of 100 $\mu g\ ml^{-1}$. The LST medium was then dispensed into tubes containing Durham vials and the tubes were sterilized in an autoclaved.

Analysis of contaminated samples: The efficiency of LST-MUG medium in the detection of *E.coli* was examined by using three tubes MPN method. Tap water samples were collected from many area in local city of Bangladesh. Water samples were three dilution (10, 1 and 0.1 ml) used to inoculate LST-MUG medium. Each assay was setup in triplicate (9 tubes per dilution) and incubated as specified previously (Anon, 1989). Presumptive counts of coliforms (gas production) and *E.coli* (fluorescence) were determined. The tubes that were gas positive [gas (+)], fluorescence positive [fluorescence (+)], or both were sub cultured into EC broth and incubated at 45^0C to confirm the presence of fecal coliforms. A comparative fecal coliform count for each sample was also obtained by using standard conventional method (Anon.1989). Raw milk samples were collected from Dairy farm Each sample was analyzed in triplicate, by using the three tubes MPN assay with LST-MUG medium. Feng and Hartman (1982) developed the rapid assay method.

Identification of samples: Samples were identified based on their gross morphology and cultural characteristics along with biochemical reaction pattern. The biochemical tests were performed according to the method proposed by Edwards and Ewing (1986).

Results

The presence of GUD in *E.coli* bacteria was examined by UV light. The results were observed within 24 h. The *E.coli* colonies of LST-MUG medium show the bluish color (Fig. 1) when exposed to UV light at 366nm. 4-methylumbelliferone released by GUD activity diffused into the surrounding medium and eventually covered the entire area. On the studied of 88 tap water samples and 75 raw milk samples in which LST-MUG tubes that showed gas positive (76%), fluorescence positive (68%) and gas positive (87%), fluorescence positive (73%) respectively (Table 1).

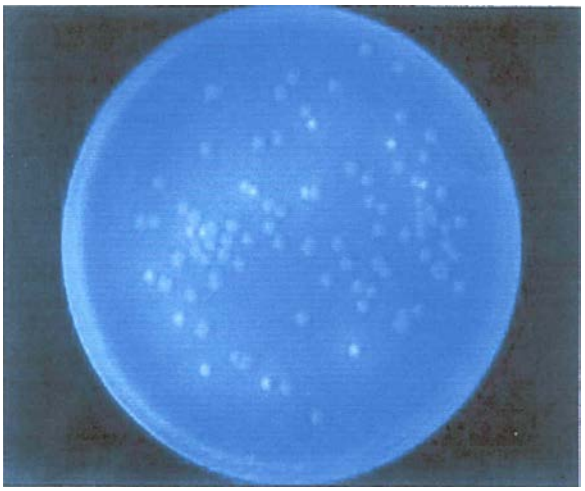

Fig. 1: The *E. coli* colonies of LST-MUG agar medium show the bluish color when exposed to UV light at 366 nm

Fig. 2: The *E. coli* colonies of LST-MUG broth shows the bluish color of middle tube under long wave UV light

Table 1: Results of tap water and raw milk analysis by using the LST-MUG MPN method

Samples	No. of tested	LST-MUG reaction	
		Gas (%)	Fluorescence (%)
Tap water	88	6.02 x 10^2 (76)	5.36 x 10^2 (68)
Raw milk	75	5.86 x 10^2 (87)	4.92 x 10^2 (73)

Table 2: Results of EC broth fecal coliform confirmatory tests on LST-MUG tubes obtained from MPN analysis of water and raw milk samples

Samples	LST-MUG reaction	NO. of tested	Positive EC broth test
Tap water	Gas (+) Fluorescence (+)	63	59 (94%)
	Gas (+) Fluorescence (-)	15	1 (7%)
	Gas (-) Fluorescence (+)	10	0 (0%)
Raw milk	Gas (+) Fluorescence (+)	59	54 (91%)
	Gas (+) Fluorescence (-)	13	2 (15%)
	Gas (-) Fluorescence (+)	3	0 (0%)

Table 3: Results of Comparative efficiency of the LST-MUG method versus conventional method for the detection of *E.coli* from MPN analysis

Samples	LST-MUG method (%)	Conventional method (%)	Variation(%)
Tap water	75 (71)	80 (76)	5
Raw milk	79 (81)	74 (75)	6

Table 4: Biochemical – physiological behavior of *E. coli* (Edwards and Ewing, 1986)

Test	Reaction
Gram negative, short, rod.	+
Indole	+
Methyl red	+
Voges-Proskauer	+
Lactose	+
H_2S	-
Urease	-
Citrate	-
Glucose, gas	+
Fermentative (TSI)	+
Cytochrome oxidase	-
Nitrate reduction	+

When MUG was incorporated into LST the presence of *E.coli* was detected by the appearance of fluorescence throughout the entire tube. *E.coli* produce gas in the tubes when inoculated into LST-MUG and shows bluish color under UV long wave of 366 nm confirming immediately that *E.coli* was present (Fig. 2).

Table 2 shows the results of EC broth fecal coliform confirmatory test on LST-MUG tubes obtained from MPN analysis by using tap water and milk samples. All tubes are subculture to EC broth and incubated at 45^{0}C to check for the presence of fecal coliform or *E.coli* (fluorescence). In tap water samples where 88 tested samples showed gas positive fluorescence positive (94%) and also observed EC confirmatory tests were positive. But there also was a 7% apparent false positive reaction (production of gas in EC medium in the absence of fluorescence), 10-gas negative fluorescence positive tubes failed to produce gas in the EC confirmatory test (apparent false negative). In 75 raw milk samples, 59 of the gas (+) fluorescence (+) tubes obtained *E.coli* confirmed (91%) by EC broth. But there also was a 15% apparent false positive reaction (Production of gas in EC medium in absence of fluorescence positive tubes failed to produce gas in EC confirmatory test (apparent false negative).

Table 3 shows the results in comparative study of conventional and rapid method. Although the conventional method is time consuming but it is a standard method where the results varied 5-6% with rapid method and it is very low percentage in the aspect of microbial study of food and food related product.

The all tubes are identified from biochemical test for further confirmation of *E.coli* (Table 4).

Discussion

The presence of GUD in bacteria has been examined by several workers Kilian and Bulow (1979) tested 633 Enterobacteriaceae strains and reported that 97% of *E.coli* are positive for GUD reaction. The possibility of incorporating MUG directly in to conventional coliform assay was tested. This idea was appealing because the efficiencies of these methods have already been established. Furthermore, coliform media were commonly used, commercially produced and therefore readily available.

The LST-MUG media in an MPN assay, a presumptive coliform count could be obtained based on gas formation and immediate conformation for *E.coli* could be obtained based on fluorescence. Non-gas forming strains comprise about 5% of the *E.coli* population (Edwards and Ewing. 1972), and they're a cause of false negative reaction in the presumptive confirmed, and even completed MPN analysis (Anderson, *et al*., 1980).

In rapid detection by incorporating MUG in coliform assays, *E.coli* may be detected immediately in one step procedures. Additional confirmatory test or biochemical reactions are not necessary, which means considerable savings in labor, time and media. Infect, if only an MPN for *E.coli* is desired. Durham tubes can be omitted from LST-MUG broth and the tubes can be examined only for fluorescence.

The analysis of tap water and raw milk samples showed that the fluoregenic LST-MUG MPN assay for *E.coli* was more efficient than the conventional method. The EC broth test for fecal coliforms confirmed that approximately 93% of the gas (+) fluorescence (+) tubes contained *E.coli*, and 7% apparently false positive reactions was observed. These results were not unusual because Geldreich (1966) reported that about 90% of *E.coli* and 8% of nonfecal coliforms produced gas in the EC broth test. The most interesting aspect, however, was that 13 gas (-) fluorescence (+) tubes failed to produce gas in EC broth confirmatory test.

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