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Evaluation of a Fluorogenic Assay for Detection of *Escherichia coli* in Foods

BARBARA J. ROBISON†

Ross Laboratories, Columbus, Ohio 43216

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A fluorogenic assay procedure with 4-methylumbelliferyl-β-D-glucuronide incorporated into lauryl sulfate broth was evaluated to detect and confirm the presence of *Escherichia coli* in foods. Fluorescence is indicative of the presence of *E. coli*; extensive biochemical confirmation is unnecessary with this assay. The 4-methylumbelliferyl-β-D-glucuronide assay was tested concurrently with our present methodology for detection of *E. coli* on 270 samples of raw ingredients and powdered food products. Total agreement between the two methods was 94.8%; there was a false-positive rate of 4.8% and no false-negatives. We found the 4-methylumbelliferyl-β-D-glucuronide assay to be rapid, accurate, simple to perform, and inexpensive.

The presence of *Escherichia coli* in food and water has long been used as an indication of insanitary conditions. Detection of this organism in foods and dairy products is usually accomplished by a most-probable-number technique (1). Tubes showing gas formation must then be confirmed as *Escherichia coli* by a series of tests, including indole, methyl red, Voges-Proskauer, citrate (IMViC) and other biochemicals. These procedures are laborious, expensive, and time consuming.

In our laboratory, the specification for *Escherichia coli* in most raw ingredients and powdered food products is that the test be negative in 11 g of sample. Therefore, the method used is qualitative rather than quantitative and involves a preenrichment, followed by selective enrichment in 2% brilliant green bile broth (BGB). Tubes showing gas formation are streaked on eosin methylene blue (EMB) agar, and suspicious colonies are confirmed as *Escherichia coli* by a series of biochemical tests. Final identification may take as long as 7 days.

Feng and Hartman (7) recently described several rapid assay procedures which utilized the substrate 4-methylumbelliferyl-β-D-glucuronide (MUG). This substrate is broken down by the enzyme β-glucuronidase to release 4-methylumbelliferone, which fluoresces under longwave UV light. Their assay is based on the fact that most strains of *Escherichia coli* (96 to 97%) produce β-glucuronidase, whereas the only other enteric bacteria which are known to produce this enzyme are some *Salmonella* (7, 11), *Shigella* (7, 9), and *Yersinia* species (P. A. Hartman, personal communication). Since all of these organisms are equally objectionable, the specificity of the assay is not severely compromised. The presence of fluorescence is considered to be a positive *Escherichia coli* test; no further confirmation is required.

The present study was undertaken to evaluate the MUG assay for detection of *Escherichia coli* in foods, both in raw ingredients and finished products, and to compare it with our current methods.

MATERIALS AND METHODS

Media. All media were commercial products obtained from BBL Microbiology Systems, Cockeysville, Md., and Difco Laboratories, Detroit, Mich. MUG was obtained from Sigma Chemical Company, St. Louis, Mo., and from Hach Company, Ames, Iowa. Lauryl sulfate broth (LSB) was supplemented with MUG to obtain a final concentration of 100 μg/ml for the study. The MUG was dissolved in warm water before addition to the LSB as recommended by Feng and Hartman (7).

Samples. The samples used in the study consisted of powdered raw ingredients and food products which were submitted to the laboratory for routine microbiological analysis. The raw ingredients tested consisted of soy protein isolates, soy protein hydrolysates, various natural and artificial flavors, caseinates, rice flour, whey protein concentrates, egg white solids, fructose, dietary fiber sources, whole egg solids, lactic acid, corn syrup solids, starches, sodium saccharin, and gelatin. Food products tested included various infant formulae (foreign and domestic), special dietary formulae, elemental diets, various frozen desserts, cereals, powdered soup, and powdered athletic beverages.

Testing procedure. All powdered carbohydrate samples were rehydrated by dissolving 11 g of material in 99 ml of sterile nutrient broth. All other samples were rehydrated in sterile phosphate-buffered water. Samples which were difficult to solubilize were homogenized for 1 min at high speed in a sterile Waring blender. All samples were then incubated at 37°C for 18 to 24 h. After incubation, the samples were tested by the current *Escherichia coli* procedure and the MUG procedure. For the current procedure, 1 ml of sample was transferred to a tube of BGB containing a Durham fermentation vial. The BGB was incubated for 24 h at 37°C. Tubes showing gas production were streaked on EMB agar. Typical colonies were then selected and inoculated into IMViC media, as well as malonate, arabinose, adonitol, glucose, inositol, sorbitol, and lactose broths, Moeller decarboxylase broths with lysine, arginine, and ornithine, motility medium, and urea and phenylalanine agars. In addition, suspicious isolates were streaked on Trypticase soy agar, and isolated colonies were used to inoculate API 20E identification strips (Analytab Products, Plainview, N.Y.). The strips were read after 18 to 24 h of incubation; conventional biochemicals were read up to 4 days. For the MUG procedure, 1 ml of sample was also transferred to a tube of LSB-MUG containing a Durham fermentation vial. The LSB-MUG was incubated at 37°C for 24 h and then was observed for fluorescence by shining a longwave UV light source (UV Blak-Ray; American Scientific Products, McGaw Park, Ill.) on the tubes. A positive LSB-MUG control tube inoculated with *Escherichia coli* (ATCC 25922) and a negative control tube inoculated with *Enterobacter*

† Present address: Litton Bionetics, Charleston, SC 29405.

cloacae (ATCC 23355) were included with each set of samples. Presence or absence of gas or fluorescence, or both, was recorded for each sample.

RESULTS

Comparison of the MUG assay and the current procedure. A total of 270 samples were tested for the presence of *Escherichia coli*. Of these 270 samples, 112 (41.5%) contained coliforms, as indicated by gas formation in the Durham vial. Total agreement between the two methods was 256 of 270 or 94.8%. Eight samples were found to contain *Escherichia coli* by both methods; one sample showed fluorescence in the LSB-MUG tube, but it was unable to be confirmed as *Escherichia coli* due to the presence of another enteric bacterium, *Enterobacter sakazakii*. This organism is basically a yellow-pigmented *Enterobacter cloacae* (10, 13), and like other *Enterobacter* species, it is capable of producing a viscous, slimy capsular material on EMB agar which can mask other organisms present in the sample (6). The natural reservoir of this organism is unknown, but it probably comes from the feces of humans and animals, sewage, water, or soil (15). The *Enterobacter sakazakii* colony isolated from the sample was inoculated into LSB-MUG, and no fluorescence was seen. This sample was therefore considered to be positive for *Escherichia coli*, a result which would have gone undetected with the conventional procedure.

Thirteen samples fluoresced in LSB-MUG and produced gas in BGB but showed no typical colonies when subcultured on EMB agar. Twelve of these samples were soy proteins, and one was a dietary fiber sample. The organism isolated from these tubes was a gram-positive coccus in pairs and short chains which produced tiny pinpoint green-sheen colonies on EMB agar. This organism was not identified, but it is believed to be a streptococcus. Littel (M.S. thesis, Iowa State University, Ames, 1982) has recently shown that some species of streptococci are capable of producing fluorescence from the MUG substrate. These false-positives (4.8% of the samples) can be eliminated if desired by streaking positive fluorescent tubes on EMB agar. If no typical colonies are present and gram-positive cocci are recovered, then a false-positive result is confirmed. However, this adds an extra day to the testing procedure. The presence of false-positives depends on the type of food being tested; laboratories considering this procedure should therefore examine the foods they test for possible false-positive reactions.

All samples found to contain *Escherichia coli* by the conventional procedure were also positive in the MUG assay, i.e., there were no false-negatives. The MUG assay was completed 48 h after test initiation, whereas a confirmed *Escherichia coli* result by the conventional procedure took 5 to 7 days.

Identification of other coliforms. Sixteen isolates which gave typical colonies on EMB agar but were MUG negative were identified by API 20E identification strips, as well as by conventional biochemical tests. Seven of the isolates were identified as *Escherichia hermannii*, a newly named organism (4) which has an IMViC pattern of ++−− and gives biochemical reactions which are very similar to those of *Escherichia coli*. Three isolates were identified as *Enterobacter agglomerans*, one as *Citrobacter freundii*, one as *Klebsiella ozaenae*, one as *Klebsiella oxytoca*, and three as *Escherichia vulneris*, another newly named organism (5) very similar to *Escherichia coli*. All other coliforms isolated from the samples did not give typical colonies on EMB agar and were not further identified.

DISCUSSION

The MUG assay for detection of *Escherichia coli* is a simple, straightforward method, and it performed very well in our laboratory. Samples containing *Escherichia coli* were detected equally well by both the conventional procedure and the MUG assay. Over 40% of the samples contained coliforms, 8.8% of which required further biochemical testing by the conventional procedure, but which were positive or negative after only 48 h with MUG. Not only are the results available earlier, but less labor is involved, because neither streaking of plates nor inoculation of biochemicals or other media is required. There was a low false-positive rate (4.8%), and there were no false-negatives. The presence of false-positives, which appeared to be due to streptococci, may depend on the types of samples tested. All of the false-positives in this study were found in soy protein or plant materials.

Two *Escherichia coli* strains recovered from the samples tested produced less enzyme than others, resulting in weak fluorescent reactions after 24 h of incubation. These tubes were reincubated for an additional 6 to 8 h, and the fluorescence was more easily detected. There was no difficulty in reading the tubes with the unaided eye.

The MUG assay offers several advantages over the conventional test procedure for *Escherichia coli*. The sensitivity of the assay is excellent. Feng and Hartman (7) showed that one viable cell present initially will produce fluorescence in 20 h. Anaerogenic strains, which account for ca. 5% of the *Escherichia coli* strains (6), are not detected by conventional procedures which depend on lactose fermentation. The MUG assay is capable of detecting these strains. Detection of enterotoxigenic and enteropathogenic strains is also difficult with conventional tests (2, 12), but Feng and Hartman (7) found the 10 enterotoxigenic strains they tested to be positive for fluorescence.

Another positive aspect of the MUG assay is its ability to detect *Escherichia coli* in the presence of other coliforms, such as *Enterobacter sakazakii*. The copious quantities of capsular material produced by this organism can prevent the isolation of any other organisms on EMB agar, yet there is no inhibition of fluorescence in LSB-MUG. There was one sample which was positive for fluorescence in LSB-MUG that could not be confirmed for the presence of *Escherichia coli* because of interference from *Enterobacter sakazakii*. However, this same sample had been tested on a previous occasion, and *Escherichia coli* was isolated at that time. To further investigate this situation, 12 samples known to contain *Enterobacter sakazakii* were inoculated with heat-stressed *Escherichia coli* at the level of 10 organisms per g. The samples were tested by using both the conventional method and the MUG assay. Gas was present in all BGB tubes, but typical *Escherichia coli* colonies were present on only four of the EMB plates; *Enterobacter sakazakii* covered the others. However, all 12 of the LSB-MUG tubes showed gas and fluorescence. A similar situation could exist with other capsule-producing organisms, such as *Enterobacter cloacae* or *Klebsiella* species.

Other organisms, such as *Proteus vulgaris*, are capable of suppressing gas formation by *Escherichia coli* in lactose-containing media (3, 8, 14). The presence of these organisms in a sample could result in a false-negative test for *Escherichia coli* by conventional procedures. As Feng and Hartman (7) demonstrated, the presence of *P. vulgaris* did not affect the production of fluorescence in the MUG medium, and *Escherichia coli* was detected even though gas production was

suppressed. Although none of the samples which we tested contained *P. vulgaris*, this organism could be present in some foods.

The cost of performing the MUG assay is also much less than that for conventional testing. A tube of LSB-MUG costs only about $0.40. On the other hand, an API 20E identification strip is ca. $2.60, and identification by means of conventional biochemicals approaches $11.00.

An interesting sidelight of the evaluation of the MUG assay was the discovery of the presence of *Escherichia hermannii* and *Escherichia vulneris* in several of the samples. The characteristics of *Escherichia hermannii* especially mimic those of *Escherichia coli*. It produces a typical green sheen on EMB, gives an IMViC of ++−−, and has similar biochemical reactions. In fact, the only biochemical tests which definitely separate *Escherichia hermannii* from *Escherichia coli* are positive KCN and cellobiose tests and a negative D-sorbitol test. In addition, *Escherichia hermannii* is yellow pigmented; most *Escherichia coli* strains are not. *Escherichia hermannii* was classified into the genus *Escherichia* based on DNA relatedness and was given its name in 1982 (4). The organism has been isolated primarily from clinical sources, and three strains have been isolated from food. However, the Centers for Disease Control believe that it is a rare species which may cause human disease (4). In the samples tested in this study, *Escherichia hermannii* was isolated from corn syrup solids, caseinates, and whey protein. *Escherichia vulneris*, also recently named (5), produces green-sheen colonies on EMB and gives an IMViC of −+−−. Reactions for arginine dihydrolase, KCN, malonate, and cellobiose separate *Escherichia vulneris* from *Escherichia coli*. Some strains also produce a yellow pigment. The organism has been isolated primarily from wounds, with one strain isolated from the environment (5). In this study, all three isolates of *Escherichia vulneris* came from corn syrup solids. For a comparison of these organisms with *Escherichia coli*, see Table 1. The finding of these two organisms in raw ingredients indicates that they may be more common than is currently thought. It is also obvious that a simple IMViC test is no longer sufficient to separate *Escherichia coli* from other similar organisms.

Because these organisms were MUG negative and were quite similar to *Escherichia coli* and little was known of their pathogenicity, further characterization of their toxin production capability was desirable. Specifically, we were concerned about the ability of these two organisms to produce the labile (LT) and stable (ST) toxins made by *Escherichia coli*. These isolates of *Escherichia hermannii* and *Escherichia vulneris* were sent to Steven Moseley of the National Animal Disease Center in Ames, Iowa. He performed colony DNA hybridizations on the organisms, which included gene probes for the LT and STb genes and two probes for the STa genes. All of the cultures were negative for each of the probes. Since the organisms were incapable of producing these toxins, their importance at this time is considered minimal.

Of course, LSB-MUG can also be used in a most-probable-number test to enumerate and confirm the presence of *Escherichia coli* in foods. Although no most-probable-number tests were included in our study, the Del Monte Corporation has conducted similar studies using the most-probable-number technique in their laboratories. They tested 1,297 samples and found a low percentage of false-positives (1.4%, all gram-positive cocci of the genus *Staphylococcus*) and no false-negative results (L. J. Moberg, personal communication). Of the samples tested, 123 contained *Escherichia coli* (9.5%). A collaborative study is being planned to obtain Association of Official Analytical Chemists approval for this assay in the near future.

TABLE 1. Comparison of *Escherichia hermannii, Escherichia vulneris, and Escherichia coli*[a]

Test	*E. hermannii*	*E. vulneris*	*E. coli*
Indole	+	−	+
Methyl red	+	+	+
Voges-Proskauer	−	−	−
Citrate	−	−	−
Lysine decarboxylase	[−]	[+]	[+]
Arginine dihydrolase	−	V	[−]
Ornithine decarboxylase	+	−	V
Growth on KCN	+	[−]	−
Malonate	−	[+]	−
Cellobiose	+	+	−
Sorbitol	−	−	+
Yellow pigment	+	V	−

[a] Symbols: +, 90 to 100% positive; [+], 75 to 89% positive; V, 26 to 74% positive; [−], 11 to 25% positive; −, 0 to 10% positive.

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