A Research Note

Evaluation of the β-Glucuronidase Substrate 5-Bromo-4-Chloro-3-Indolyl-β-D-Glucuronide (X-GLUC) in a 24-Hour Direct Plating Method for Escherichia coli

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ABSTRACT

A 24-h direct plating method for Escherichia coli using Peptone-Tergitol agar was used to compare the effectiveness of the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-GLUC) with the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) for β-glucuronidase activity. Values obtained for enumeration of two strains of E. coli recovered from artificially inoculated raw minced chicken (i.e., plating efficiencies on the inoculum, cells per g, and recovery percentages related to those on Plate Count Agar) indicate that X-GLUC at 50 μg/ml was as effective as MUG in an agar medium. Unlike MUG, X-GLUC does not require ultraviolet light illumination, and the color reaction produced remains localized in the positive colonies.

β-Glucuronidase activity is used as a rapid and relatively specific indicator of Escherichia coli in many foods and environmental samples. The fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) (5) is an efficient indicator of enzyme activity. The addition of MUG to lauryl tryptose broth by Feng and Hartman (2) improved both the speed and sensitivity of the standard most probable number (MPN) method. Recently, Damare et al. (1) developed a 24-h direct plating medium containing MUG and also Tergitol 7 to inhibit gram-positive bacteria. In this study we have evaluated the new chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-GLUC) (3) as a substitute for MUG in a 24-h plating assay. When added to the basal medium of Damare et al. (1), we found that X-GLUC was as effective as MUG in recovering E. coli from artificially inoculated minced chicken, and in addition, had certain advantages over MUG in the enumeration of colonies on agar surfaces.

METHODS AND MATERIALS

Stationary phase cultures of E. coli (two strains isolated from ground beef -- F1113 and F1114) were grown for 24 h in Brain Heart Infusion Broth (Difco, Detroit, MI) at 37°C before resuspension in an equal volume of 0.85% saline solution. After 454 g of thawed minced chicken were inoculated with 10 ml of a 1:10 dilution of the suspended cells in saline solution, three 25-g samples were removed and each was blended in 225 ml of 0.1% peptone water for 2 min. All dilutions for spread-plating in triplicate were made in 0.1% peptone water. Peptone-Tergitol Agar (PTA) (1) was used as the base for preparation of both the MUG-containing agar (PTG) (7) and the X-GLUC-containing agar (PTX). A stock of X-GLUC (Research Organics, Cleveland, OH) prepared by dissolving 20 mg in 1 ml of dimethylformamide (DMF) (Sigma Chemical Co., St. Louis, MO) was added without sterilization to the tempered agar base at a concentration of 50 μg/ml. All plates were incubated at 35°C and observed 16-24 h after inoculation. In addition to PTG and PTX media, appropriate dilutions of both the inoculum and the inoculated chicken were also plated on Plate Count Agar (PCA) (Difco, Detroit, MI) and added to Lauryl Sulfate Broth (LSB) (BBL, Cockeysville, MD) for a 3-tube MPN determination at 35°C. Positive MPN tubes were inoculated into EC Broth (Difco, Detroit, MI) and incubated at 44°C for 48 h.

RESULTS AND DISCUSSION

To determine the optimal concentration of X-GLUC in PTA, a series of plates containing X-GLUC at concentrations up to 50 μg/ml were spread with E. coli and observed after 24 and 48 h. Maximal color intensity was obtained in 24 h at 50 μg of X-GLUC/ml, thus agreeing with a previous report of its use in a different medium (3). Color intensity was almost as vivid at 40 μg/ml; however, at lower concentrations (20 or 30 μg/ml) the intensity was reduced perceivably and color development was not detectable as early. Neither X-GLUC nor its solvent DMF produced any discernible inhibition of colony size or number at the levels used in this study -- or even at DMF levels over 6 times greater (data not presented). All dilutions, therefore, were plated on PTX agar containing 50 μg of X-GLUC/ml. Enumeration of the two strains of E. coli recovered from artificially inoculated raw minced chicken using PTG agar and PTX agar is summarized in Table 1. Counts obtained on a nonselective medium, PCA, and from the MPN assay on one strain are also included as well as plating efficiencies and recovery percentages. These data show very similar recovery values on all the media used, with good agreement between the PTX and PTG media.
indicating that X-GLUC is as effective as MUG under the experimental conditions used in this study. However, because the food samples were inoculated with E. coli at levels approximately 200 times the original total plate count (Table 1), direct comparisons and quantitation of recoveries on the various media were made without interference from the normal flora. In contrast, at the lower dilution levels used to enumerate E. coli in naturally contaminated foods, recovery of essentially pure cultures of E. coli would normally be precluded by the predominance of other bacteria. Thus on media containing Tergitol 7 (PTG and PTX), which are selective for total gram-negative bacteria, E. coli in naturally contaminated foods would usually represent only a small proportion of the total gram-negative population. Under these conditions E. coli can be differentiated on PTG and similarly selective media by the presence of a p-glucuronidase indicator such as MUG (1,6). The question remains, however, as to the validity of comparing directly the enumeration of E. coli on these types of media with the more conventional lactose-based media capable of identifying total presumptive coliforms by acid and/or gas production. Damare et al. (1), studying meat and poultry samples, made such a direct comparison and concluded that E. coli counts enumerated on PTG were equivalent or better than counts made using a 5-tube MPN procedure. Alternatively, Petzel and Hartman (6), using a MUG-containing agar selective for total gram-negative bacteria, concluded that because of the high background of other gram-negative bacteria and the relatively low numbers of E. coli, the medium was useful in determining only the approximate level of E. coli in most food samples.

The relative specificity of β-glucuronidase activity as being indicative of E. coli has been established in previous studies. Over 95% of E. coli strains are positive for β-glucuronidase (1,2) with strains of Shigella and Salmonella registering about 50 and 30% positive activity, respectively. Certain strains of some other members of the Enterobacteriaceae have also been reported to exhibit false-positive activity in 24 h, but at a much lower incidence (1,2,6,7). Foods containing endogenous β-glucuronidase such as oysters (mollusks), snails, and fish liver, however, might be expected to contribute problems in E. coli assays based on detecting this enzyme (4).

The principal advantages of X-GLUC over MUG as an indicator of β-glucuronidase activity are: (a) colony enumeration without using ultraviolet light illumination, and (b) limitation of the blue color reaction to the positive colonies instead of, as with MUG, a diffusion of fluorescence over the entire agar surface by 24 h complicating colony differentiation. Although these two advantages alone should make X-GLUC more suitable for routine laboratory manipulations, particularly to extended incubation periods, there are several disadvantages to its use.

Currently, the use of X-GLUC in routine plating media has limited practicality because of its high cost (about 25 times the cost of MUG). It is potentially cost-effective in a filtration medium, however, where as little as 3 ml of PTX will support the growth of E. coli on membrane filters (data not presented).

Aside from cost considerations, X-GLUC is also an unsuitable indicator in the liquid LSB medium because there is insufficient color intensity developed in 24 h or longer even at 100 µg/ml (data not presented). Finally, unlike the fluorogenic substrate MUG, the color produced by X-GLUC in β-glucuronidase-positive colonies could interfere with either additional differential or confirmatory reactions involving color changes.

Although X-GLUC works well in an agar medium in recovering E. coli from artificially inoculated food, further work is required to evaluate its enumeration potential in a variety of media and naturally contaminated food products. Modifications in the selective medium containing X-GLUC could lead to further improvements in the overall 24-h procedure used in this study.

### TABLE 1. Enumeration of Escherichia coli from inoculated raw minced chicken on different media.

<table>
<thead>
<tr>
<th>Medium(^a)</th>
<th>E. coli strain(^c)</th>
<th>Cells per g(^d) (×10^6)</th>
<th>Plating efficiency (%)(^e)</th>
<th>Recovery (%)(^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA</td>
<td>F1113</td>
<td>5.83</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>F1114</td>
<td>4.73</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>5.28 ± 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTG</td>
<td>F1113</td>
<td>6.03</td>
<td>120</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>F1114</td>
<td>5.71</td>
<td>91</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>5.87 ± 0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTX</td>
<td>F1113</td>
<td>5.53</td>
<td>119</td>
<td>95</td>
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<tr>
<td></td>
<td>F1114</td>
<td>5.18</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>5.36 ± 0.175</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSB (MPN)</td>
<td>F1113</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>F1114</td>
<td>5.0</td>
<td>103</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^a\)The natural contamination level of the chicken was 2.65x10^4 cells/g - well below the recovery level at the dilutions used.

\(^b\)See Methods and Materials for media descriptions.

\(^c\)E. coli strains F1113 and F1114 were isolated from raw ground beef using selective and differential media and identified using the Enterotube II system. They were also determined to be β-glucuronidase-positive on PTX and PTG plates.

\(^d\)Represents the average value of three 25-g samples from each experiment plated at appropriate dilutions in triplicate.

\(^e\)Calculates by enumerating the inoculum separately on each medium divided by its enumeration on PCA times 100.

\(^f\)Based on average number of cells recovered per g versus the number of cells recovered per g on PCA.
ACKNOWLEDGMENTS

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REFERENCES

8. Farber et al., con't. from p. 401