Comparison of β-Glucuronidase and Indole-Based Direct Plating Methods for Enumerating Escherichia coli in Artificially Inoculated Ground Meats

ELON W. FRAMPTON*, LAWRENCE RESTAINO, and NANCY BLASZKO
Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115

(Received for publication February 26, 1990)

ABSTRACT

Peptone tergitol glucuronide (PTG) agar containing 4-methylumbelliferyl-B-D-glucuronide (MUG) (for β-glucuronidase activity), the Holbrook, Anderson, Baird-Parker (HABP) method (for detecting indole production), and the standard 3-tube most probable number (MPN) method were compared with plate count agar (PCA) for enumerating three strains of unstressed Escherichia coli artificially inoculated into ground beef and chicken at 1-6 x 10^6 cells/g. No significant difference (P>0.05) was determined between PTG agar and PCA in the recovery of E. coli. The MPN method enumerated a significantly greater (P<0.05) number of E. coli cells than PCA. Compared with PCA, the HABP method recovered a significantly lower (P<0.05) number of E. coli cells from chicken, whereas no significant difference (P>0.05) was obtained with ground beef. When combining all data from chicken and beef, the recovery of E. coli cells by the HABP method was also significantly lower (P<0.05). Overall, based on the enumeration of E. coli on PCA, the HABP method, PTG agar, and MPN method recovered 57, 102, and 144%, respectively.

As alternatives to the standard most probable number (MPN) method, several direct and rapid plating procedures for enumerating Escherichia coli within 24 h have been developed and tested recently in a variety of foods (7,8). The fluorogenic substrate 4-methylumbelliferyl-B-D-glucuronide (MUG) for detecting β-glucuronidase activity has been incorporated into lauryl sulfate tryptose (LST) broth (5), peptone tergitol glucuronide (PTG) agar (3), violet red bile (VRB) agar (1), and M-Endo broth (1) used in membrane filter plates, for an accurate and rapid enumeration E. coli in various food products.

A direct plating medium for enumerating E. coli within 24 h based on indole production was developed by Anderson and Baird-Parker (2). Using 248 food samples, the enumeration of E. coli on this medium compared favorably with the MPN method with 95% of the E. coli identified as biotype 1 (2). A subsequent modification of this method described by Holbrook, Anderson, and Baird-Parker (HABP) added a resuscitation step before the cells were exposed to the selective tryptone bile agar (TBA) at 44 ± 0.5°C (10). This modification allowed injured cells to recover before exposure to the added stress of a selective medium and higher temperature and improved the enumeration of E. coli from naturally contaminated frozen meat samples (10).

The purpose of this investigation was to compare the PTG agar method (MUG substrate) with the HABP procedure (indole production) in the recovery of E. coli from artificially inoculated ground beef and chicken. The standard used for comparison was based on the enumeration of E. coli on nonselective plate count agar (PCA). Additionally, all plating methods were compared with values obtained using the standard 3-tube MPN procedure. Minced beef and chicken artificially inoculated with E. coli (between 1 and 6 x 10^6 cells/g) at levels exceeding the total plate count of these meats were used to compare these methods.

MATERIALS AND METHODS

Stationary phase cultures of E. coli strains (ATCC 25922 and two strains isolated from ground beef Fl113 and Fl114) were used in this study. All strains were β-glucuronidase positive. Cultures were maintained on nutrient agar slants by monthly transfers and stored at 4°C. The E. coli strains were grown for 24 h in brain heart infusion broth at (BHIB) 37°C without agitation. After incubation, 1 ml of the cell suspension was transferred to 100 ml of BHIB and incubated for an additional 24 h at 37°C. The inoculum was centrifuged in sterile polycarbonate bottles at room temperature for 30 min at 3,300 x g. The pellet was resuspended in 100 ml of sterile 0.85% saline and adjusted to an absorbancy between 0.35 and 0.5 at 600 nm in a Spectronic 20 spectrophotometer using 18 x 150 mm tubes.

Frozen minced raw beef and chicken were thawed for 24 h at 4°C before inoculation. A 454-g portion of thawed chicken or beef was inoculated with 10 ml of a 1:10 dilution of the resuspended cells. The inoculum was aseptically dispersed in the meat by mixing manually for 5 min. Three 25-g samples were removed and blended in 225 ml of 0.1% sterile peptone for 2 min.

Total viable cells in both the inoculum and the inoculated meat samples were enumerated on PCA. The PTG agar containing the substrate MUG (Sigma Chemical Co., St. Louis, MO) at 100 μg/ml was prepared as described by Damaré et al. (3). The HABP method (10) used minerals modified glutamate agar (MMGA) as the resuscitation medium. MMGA was prepared from Oxoid's MM medium base per manufacturer's instructions. Cellulose acetate membranes (Nuflow, Oxoid Ltd., Basingstoke, England, 85 mm diam, 0.45 μm pore size) were aseptically placed (shiny side down) and flattened with a sterile bent glass rod to previously well-dried (2 h at 35°C without lids) MMGA plates. One-half mL volumes were spread on each MMGA plate, whereas 0.1 mL volumes were used for all other spread plating procedures. For the 3-tube MPN method, standard procedures (14) were employed. All solid media

JOURNAL OF FOOD PROTECTION, VOL. 53, NOVEMBER 1990
were inoculated in triplicate whereas the 3-tube MPN method was performed in duplicate. All serial dilutions were made in 0.1% peptone.

PCA plates were incubated at 35°C for 48 h. The PTG agar plates were incubated at 35°C for 16-24 h before identifying fluorescent colonies under longwave (365 nm) ultraviolet light. For the resuscitation step on MMGA, plates were incubated at 37°C for 4 h before transferring the membranes to TBA plates for an additional 18-24 h at 44°C. After incubation, indole positive colonies were identified with the Vracko and Sherris (15) reagent.

The percentage recoveries of E. coli from the selective enumerating procedures were compared with the number of colonies recovered on PCA plates. With a total of 250 data points, analysis of variance computations were performed using SAS programs. Duncan’s multiple range test for variable (4) was used to calculate the 95% significance levels for E. coli on PCA versus the MPN method, PTG agar, and the HABP medium.

RESULTS AND DISCUSSION

As shown in Table 1, the enumeration of E. coli by direct plating on PTG from meats previously inoculated with unstressed cells, produced values very similar to the nonselective PCA. Average recoveries of 102% were obtained from both beef and chicken. Differences were apparent, however, when the PTG method was compared with the HABP method and the MPN procedure. Typically, the HABP method produced lower recovery values. The average values were significantly lower (P<0.05) in the combined (beef and chicken) recovery data (57%) and with the chicken alone (49%). Conversely, the average MPN values were significantly higher (P<0.05) than the PTG method for both beef and chicken with a combined recovery value of 144%. In summary, these results indicate that for recovering unstressed E. coli from artificially inoculated meats that (i) the PTG method was as effective as the nonselective medium and recovered more than the HABP method, and (ii) the MPN method overestimated the number of E. coli.

Previously, Damaré et al. (3) compared the PTG and HABP (HABP is used generically to refer to any method where a resuscitation step on any nonselective medium precedes transfer to TBA at 44 ± 0.5°C) methods on the recovery of both freeze- and heat-stressed E. coli from artificially inoculated meat samples. For freeze-stressed cells, values of 70% were reported for both methods based on the recovery on a nonselective medium (3). Although no comparison was made with the MPN method, E. coli recoveries were much lower when these freeze-stressed cells were plated directly on VRB agar and TBA with values of only 13% and less than 10%, respectively (3). Recoveries of heat-stressed E. coli were higher in all cases with values of 100% and over 90% for PTG and HABP, respectively (3). Thus, Damaré et al. (3) concluded that stressed cells are recovered efficiently on the PTG medium. Comparisons were not made with unstressed cells. The same study also reported that the PTG method provided recoveries that were equal to or better than the 5-tube MPN method when freeze-stressed E. coli was enumerated in naturally contaminated and partially thawed meat samples (3). This result was confirmed using the PTG agar base with a different β-glucuronidase indicator (13). Previously, Holbrook et al. (10) reported that the HABP method recovered a similar or greater number of sublethally damaged E. coli from various artificially or naturally contaminated foods than were recovered by a 3-tube MPN method using purple MacConkey broth. Recently, Havelaar and During (9), in a collaborative study involving eight laboratories, reported a 73% recovery of E. coli from artificially inoculated water samples using an HABP-type procedure. The samples were chilled to ice bath temperature before distribution to participating laboratories, and methods involving the direct exposure of unresuscitated cells to media containing selective agents were not applied (9). Additionally, variations in incubation temperatures between laboratories as well as in heat sensitivities of different E. coli strains were found to affect significantly the recovery values (9).

Thus, these variables together with differences in the type and degree of cell stress involved in various investigations make unequivocal comparisons between studies difficult. However, recoveries of artificially inoculated E. coli on a triplate basis (3) were not made with unstressed cells. The same study also reported that in experiments the inoculum levels on plate count agar ranged between 1x106 and 6x106 cells/g.

Differences in the combined (beef and chicken) percentage recoveries make unequivocal comparisons between studies difficult. However, recoveries of artificially inoculated E. coli strains from each in­oculum plated directly (without addition to meat) on the individual selective medium versus direct plating on PCA and averaging the results for the three strains. In this analysis, only the MPN method was significantly different (P<0.05) from the other methods.

TABLE 1. Comparison of the enumeration methods for the recovery of Escherichia coli strains artificially inoculated into minced beef and chicken.

<table>
<thead>
<tr>
<th>Strain</th>
<th>PCA</th>
<th>Enumeration media</th>
<th>MPN</th>
<th>PTG</th>
<th>HABP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 25922</td>
<td>100</td>
<td>116</td>
<td>114</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>F1113</td>
<td>100</td>
<td>167</td>
<td>95</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>F1114</td>
<td>100</td>
<td>190</td>
<td>98</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>Average recovery (%)</td>
<td>100</td>
<td>154</td>
<td>102</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

The data using strain ATCC 25922 are based on twice the number of data points obtained for the other two strains.

Numbers represent the percentage recovery for each enumeration medium calculated from the average number of E. coli cells enumerated on a particular medium divided by the average number of cells enumerated on plate count agar times 100. Each number was calculated from three samples each plated in triplicate. For all experiments the inoculum levels on plate count agar ranged between 1x106 and 6x106 cells/g.

For beef, the average value of E. coli cells enumerated by the MPN method was significantly greater (P<0.05) than the cells recovered on the other three media. PCA, PTG agar, and HABP are not significantly different (P>0.05).

For chicken, the MPN method and the HABP medium are significantly different (P<0.05) (MPN greater and HABP less) from PCA and PTG agar for the enumeration of artificially inoculated E. coli cells. The PCA and PTG agar are not significantly different (P>0.05).

Differences in the combined (beef and chicken) percentage recovery followed the same significance patterns as observed in (d) for chicken.

Calculated by dividing recovery of E. coli strains from each inoculum plated directly (without addition to meat) on the individual selective medium versus direct plating on PCA and averaging the results for the three strains. In this analysis, only the MPN method was significantly different (P<0.05) from the other methods.

JOURNAL OF FOOD PROTECTION, VOL. 53, NOVEMBER 1990
coli of approximately 70% on HABP (3,9) are generally close to the HABP recovery values of 57% from meat and 75% plating efficiency shown in Table 1. Also, the significantly greater number of E. coli enumerated by the MPN method has been reported previously (6,11,12). Usually this difference is not apparent when stressed cells are enumerated by the MPN method because of selective components in the medium.

An extension of the present study to include the recovery of sublethally injured E. coli strains from artificially inoculated meats should help to elucidate the equivalency of the two direct plating methods and the MPN procedure. Although the artificial inoculation technique provides a means of rapidly comparing the recovery efficiencies of various methods, the efficacy of any method established by this technique should also be verified on naturally contaminated foods.

ACKNOWLEDGMENTS

The statistical assistance of Drs. Sudhir Gupta and Samuel Scheiner and the technical assistance of R. Johnson, J. RochaDieu, C. Shilling, and Y. P. Tao on portions of this study is greatly appreciated.

REFERENCES