A Research Note

Organisms From Positive MPN Tubes Inoculated With Samples That Yielded No Growth on Pour Plates

J. A. KOBURGER and J. L. OBLINGER

Food Science Department, University of Florida
Gainesville, Florida 32611

(Received for publication November 8, 1976)

ABSTRACT

Twenty samples of cold ready-to-eat cereal were analyzed for total organisms using the pour plate and Most Probable Number technique (MPN). Nine samples exhibited growth in MPN tubes but not on pour plates. Of the 28 isolates obtained from the MPN tubes, 16 were members of the genus Bacillus and the remainder were predominately other gram-positive organisms. The data support the working hypothesis that aside from the mathematical bias associated with the MPN procedure, microbial recovery is more favorable in a liquid environment than in an agar medium.

In previous reports (5,6) when comparing the MPN method to the pour plate method for recovery of total organisms it had been observed that some samples exhibited growth in MPN tubes but not on pour plates. This fact was observed a sufficient number of times in our laboratory, particularly with dry cereals, to warrant further investigation. It was hoped that identification of the organisms would lead to a possible explanation of this phenomenon.

MATERIALS AND METHODS

Twenty samples of ready-to-eat cold cereal were obtained from retail stores in the Gainesville, Florida area. Plate Count agar and Plate Count broth were made from the separate ingredients and sterilized.

Serial 1:10 dilutions of the samples were made and duplicate pour plates of each dilution were prepared (7). A three-tube MPN series was also prepared at each dilution (7) and 1-ml aliquots of each dilution were used to inoculate both plates and MPN tubes. Incubation was at 20°C for 5 days.

Those samples that did not exhibit growth in any of the pour plates, but did show growth in broth were selected for further study. The growth-positive tubes were streaked onto duplicate plates of Plate Count agar, with one plate incubated aerobically and the other anaerobically in a GasPak® jar.

Representative colonies from the aerobic plates were transferred to Plate Count agar slants, whereas colonies from the anaerobic plates were picked into thioglycollate broth.

Identification of the isolates was made according to the descriptions in Identification Methods for Microbiologists (4) and the 8th edition of Bergey's Manual of Determinative Bacteriology (2).

RESULTS AND DISCUSSION

Of the 20 samples analyzed, nine samples showed growth in the broth but not on the pour plates (Table 1).

Recognizing the positive bias associated with the MPN procedure due to sample size and probability, one might expect no growth on plates but growth within tubes approximately 40% of the time. These factors might explain the tabular values of 3.6 but not the higher estimates obtained with some samples. However, our purpose was not to question the statistical determinations but rather to explain the differences in recovery from a practical standpoint. Two samples exhibited growth only under anaerobic conditions (C-4 and I-5) with only one of the two isolates, a strict anaerobe. Of the 28 isolates, 16 were members of the genus Bacillus with the remainder also being predominately gram-positive organisms. Acinetobacter calcoaceticus was isolated from one sample and it was recovered from three of the five growth-positive tubes for that sample, indicating a level of about 15 organisms per gram. Yeasts were isolated from two samples (A-2 and G-10) and were the only fungi found.

That the majority of the organisms were gram-positive is not surprising, in that they are known to be more resistant to adverse conditions such as heat and desiccation. It might be assumed that conditions for outgrowth were more favorable in a liquid environment than in a gel. This may be related to medium components, oxygen tension, formation of toxic substances between the agar and other medium constituents, etc. Additionally, conditions in a liquid environment may possibly be more favorable for germination due to the constant bathing of the spore in fresh medium during the early phases of outgrowth.

While it is not possible to establish exactly why recovery by the MPN procedure was better as opposed to direct plating, the data do point out the need for a better understanding of the recovery of microorganisms from foods and the particular needs of such organisms during...
TABLE 1. Identification of organisms recovered from growth-positive MPN tubes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Isolates</th>
<th>Dilution of sample</th>
<th>MPN/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$10^{-1}$</td>
<td>$10^{-2}$</td>
</tr>
<tr>
<td>A-2</td>
<td>6.2 Yeast, Nocardia convoluta</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>A-3</td>
<td>Bacillus megaterium, B. subtilis</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>C-4</td>
<td>Clostridium fallax</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>D-7</td>
<td>Micrococcus luteus</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>E-8</td>
<td>B. subtilis, B. megaterium, B. cereus</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>F-9</td>
<td>Corynebacterium poinsettiae, Acinetobacter calcoaceticus, B. lentus, B. licheniformis</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>G-10</td>
<td>Yeast, B. licheniformis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>H-1</td>
<td>Corynebacterium spp.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>J-5</td>
<td>M. luteus</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Number of positive tubes of three tubes inoculated

periods of resuscitation (3). Our work does, however, support the working hypothesis of many microbiologists that, at least with some foods, recovery of certain organisms appears to be better in liquid rather than solid medium and that when low levels of organisms are to be recovered, use of a procedure employing a liquid environment should be considered.

REFERENCES


