A Research Note

Comparison of VRB and VRB-2 Agars for Recovery of Stressed Coliforms From Stored Acidified Half-and-Half

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ABSTRACT

Half-and-half was acidified with delta-gluconolactone, inoculated with three species of coliform bacteria, stored for 31 days at 5 °C, and examined for numbers of viable coliforms on VRB and VRB-2 agars. Loss of culture viability was logarithmic with recovery of 50 and 10% of initial numbers on days 7 and 30, respectively. Escherichia coli had significantly more recoverable injured cells than did Enterobacter aerogenes or Klebsiella pneumoniae. As time of storage increased, the proportion of injured to noninjured cells also increased. However, the maximal number of injured cells was on the thirteenth day of storage of E. coli-inoculated product. VRB-2 agar averaged 20% higher in productivity than VRB agar.

Hartman et al. (2) developed Violet Red Bile Agar-2 (VRB-2) in an effort to recover coliform bacteria which had been stressed. Samples are plated in a basal layer of Plate Count Agar which is overlaid with double strength VRB agar. By the time inhibitors diffuse into the basal layer injured cells are claimed to have had time to repair and can grow in the presence of inhibitors from VRB agar. In a comparative study among several laboratories, VRB-2 agar was considerably more productive than VRB agar in testing raw milk, ice cream and cottage cheese (3).

The extent of injury to the bacterial cells ranges from lethal to sub-lethal. Since its degree of damage varies among individual cells, it is obvious there would be differences in recovery rates among strains of each species (5).

This study was undertaken to determine attrition rates and the percentages of injured cells among three species of coliform bacteria in directly acidified half-and-half. It was assumed that at a given time with a given sample the differences in productivity of VRB and VRB-2 media represented numbers of moderately injured cells. Cells severely injured yet recoverable on Plate Count Agar may not repair rapidly enough in the Plate Count Agar of the VRB-2 medium to escape the effects of the inhibitors. Thus they may not have formed visible colonies in these experiments.

MATERIALS AND METHODS

VRB and VRB-2 media (Difco) were hydrated in cold water at rates of 4.15 and 4.3 g/100 ml, respectively. The media were heated to boiling and cooled to 45 °C before use. Plate Count Agar was prepared according to Standard Methods for the Examination of Dairy Products (4).

Escherichia coli UMCS, Enterobacter aerogenes UMC6 and Klebsiella pneumoniae UMC7 were obtained from the University Food Microbiology collection.

Before inoculation of acidified half-and-half each test organism was grown in Trypticase soy broth for 24 h at 37 °C, and numbers of colony-forming units per milliliter were determined by plating in VRB agar according to Standard Methods (4).

Acidified half-and-half was prepared from pasteurized half-and-half plus 1.2% nonfat dry milk (NDM), 1.4% stabilizer (Vitex 73) and 1.0% acidulant (Vitex 201). Stabilizer and acidulant (delta-gluconolactone) were obtained from Vitex Division, Mallinkrodt, Inc., St. Louis, MO. Half-and-half was heated to 32 °C and the NDM and stabilizer were added while stirring. After cooling to 20 °C, coliforms were added at a rate of approximately 500/ml. The acidulant was then added. Nonacidified controls were prepared by substituting sterile water for the acidulant. Samples were stored at 5 °C for 31 days.

Counts of coliform bacteria were made on treated and control samples on days 0, 1, 3, 7, 13, 21 and 31 after sample preparation. Six plates were prepared from a 1:10 dilution of each sample, and three plates each were poured with VRB and VRB-2 agars. For the VRB-2 treatment, plates were first poured with 7-8 ml of Plate Count agar. After solidification, these plates were overlaid with 7-8 ml of VRB-2 agar. For the VRB treatment, plates were poured in the same manner except VRB agar composed both layers. All plates were incubated at 32 °C and counted after 24 h.

There were two replications of the experiment, and the data were analyzed by analysis of variance with the variables being storage time, media and coliform species. All possible interactions were tested, and the means were differentiated by Least Significant Difference Tests (7).

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RESULTS AND DISCUSSION

These experiments with acidified half-and-half showed that coliforms died at a logarithmic rate, that the strain of *Escherichia coli* tested had a higher percentage of moderately stressed cells during storage than did the strains of *Enterobacter aerogenes* or *Klebsiella pneumoniae*, and that more coliforms were recoverable in VRB-2 agar than in VRB agar.

Regression analysis of the log$_{10}$ transformed data showed that the percentage of coliforms recovered decreased logarithmically and by 90% in 30 days ($r^2 = .99$). After 7 days, overall average counts had been reduced by 50%.

Among the three species (Fig. 1A, 1B and 1C), by far the greatest percentage of moderately injured cells occurred with *E. coli*. (The difference between the solid and dashed lines in the figures represents moderately injured cells.) On day 13 nearly 60% of the initial inoculum of *E. coli* was recovered on VRB-2 agar and nearly 70% of these were moderately injured. In contrast, only 25% of *E. aerogenes* and 35% of *K. pneumoniae* were recovered on day 13, and moderately injured cells averaged about 30% with each species. Only with *E. coli* was the mean count on VRB-2 agar significantly higher ($P<.05$) than the mean count on VRB agar.

Although there were species differences in the above experiments, it is safe to conclude only that there were strain differences because we only tested one strain of each species.

Figure 1D illustrates that injured coliforms (means for the three species), as a fraction of the viable population, increased with time. On day 1 of storage, 98% of the coliforms were recovered on VRB-2 agar and about 8% were moderately injured. On day 13 about 43% were recovered on VRB-2 and slightly more than 50% of these were moderately injured. By the 31st day only 10% of the initial inoculum was recovered and about 80% of those were moderately injured. When data were averaged over the entire storage time and the three genera, VRB-2 agar recovered 20% more coliforms than did VRB agar, a significant difference ($P<0.05$).

REFERENCES