Performance of Four Selective Media for Enumerating *Staphylococcus aureus* in Corned Beef and in Cheese

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**ABSTRACT**

The performance of four selective media for enumerating *Staphylococcus aureus* in artificially contaminated samples of corned beef was strain-dependent. Baird-Parker (BP), Kranep (KR), Mannitol Salt (MS) and Staphylococcus medium 110 (S110) performed equally well in enumerating an enterotoxin A producing strain, but KR and BP were significantly better than S110 in enumerating an enterotoxin D producing strain of *S. aureus*. In naturally contaminated cheese samples which abound with competing microorganisms, BP performed significantly better than the other three media.

In their study on survival of an enterotoxin A producing strain of *Staphylococcus aureus*, Mansfield et al. (3) noted that Baird-Parker (BP) agar tended to give falsely low counts of the organism in cans of corned beef which had been stored for periods of 1 month or longer. In contrast, Kranep (KR) agar yielded counts which remained relatively constant over the entire 12-month storage period, and the counts were much higher than on BP agar.

In another earlier study, Stiles (7) found that counts of four different strains of *S. aureus* in a variety of cheeses were significantly lower on BP agar than on Mannitol Salt (MS) agar or Staphylococcus medium No. 110 (S110), and the differential increased as the ripening period of the three cheese types progressed. Rayman et al. (5) observed that counts of *S. aureus* in a variety of cheese samples were higher on BP agar than on KR agar. Neither MS nor S110 medium was used in this study, but Milk Salt agar, which is also a high salt medium, was found to be less productive than BP agar.

The differences in results prompted the present study which compares the performance of BP, KR, MS and S110 media for enumerating *S. aureus* in corned beef and in naturally contaminated cheese samples.

**MATERIALS AND METHODS**

**Cultures**

An enterotoxin A (SEA) and an enterotoxin D (SED) producing strain of *S. aureus*, both of which were isolated from foods implicated in food poisoning incidents, were used in the study. The organisms were grown in Brain Heat Infusion broth (Difco) for 24 h at 35°C, diluted in 0.1% peptone water and inoculated at two levels into cans of corned beef.

**Media**

Baird-Parker agar (Oxoid), Kranep agar (Oxoid), Mannitol Salt agar (Difco) and Staphylococcus medium No. 110 (Difco) were prepared according to the manufacturers’ instructions.

**Inoculation of corned beef**

Twelve-ounce cans of corned beef imported from Brazil were purchased locally. The tops of the cans were disinfected by swabbing with 2% iodine in 70% alcohol and placed to dry in a laminar flow hood. Silicon sealant (Canadian General Electric) was applied to the top of the can to cover an area of approximately 2.5 cm in diameter. At the center, the sealant was 0.75-cm thick. After curing for 48 h in the laminar flow hood, the sealant and top of the can were pierced with a sterile No. 13 gauge hypodermic needle through which filtered nitrogen was flowing. The needle was withdrawn and 0.1 ml of a diluted suspension of *S. aureus* cells was injected into the corned beef. For each enterotoxin-producing strain, a low inoculum of 10³ cells and a high inoculum of 10⁴ cells were injected. Sterile melted paraffin wax was poured over the sealant and aluminum foil was placed over the wax.

The inoculated cans and uninoculated controls were incubated at 30°C and at room temperature.

**Enumeration of S. aureus**

At predetermined intervals, two cans inoculated with the SEA producer at each of the two inoculum levels, and two cans inoculated with the SED producer also at each of the two inoculum levels, were removed from the 30°C incubator and analyzed for *S. aureus*. At the same time one uninoculated control from the 30°C incubator was analyzed. The sampling and analysis were repeated for cans incubated at room temperature.

The cans were opened under aseptic conditions and the entire content of each can was homogenized in 0.1% peptone water at a ratio of 1:1. Dilutions in peptone water were surface- plated in duplicate onto each of the four media and the plates were incubated at 35°C for up to 48 h. Colonies presumed to be *S. aureus* were confirmed by the tube coagulase and thermostable nuclease (2) tests.

Cheese samples were homogenized in 9 volumes of 0.1%
TABLE 1. Multiple comparisons of media for each observation timea.

<table>
<thead>
<tr>
<th>Time (Weeks)</th>
<th>SEA producing strain</th>
<th>SED producing strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BP(6.79)b KR(6.70) MS(6.65) S110(6.62)</td>
<td>KR(7.17) BP(7.14) MS(7.05) S110(6.76)</td>
</tr>
<tr>
<td>4</td>
<td>KR(7.38) BP(7.10) S110(6.98) MS(6.91)</td>
<td>BP(7.11) KR(7.07) MS(6.68) S110(5.92)</td>
</tr>
</tbody>
</table>

a Media underscored by same line do not differ significantly at a comparisonwise level of a = 0.05. Comparisons are made separately for each strain due to the presence of a significant medium x strain interaction.

b Mean log counts are in brackets.

TABLE 2. Multiple comparisonsa of media for naturally contaminated samples using Friedman rank sums.

<table>
<thead>
<tr>
<th>Highest recoveries</th>
<th>Lowest recoveries</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP(3.94)b</td>
<td>MS(3.40) S110(3.43) KR(2.72)</td>
</tr>
</tbody>
</table>

a Media underscored with same line do not differ significantly in rank at overall 5% level of significance.

b Figure in brackets is mean log count over all 13 samples.

BP=Baird-Parker agar; KR=Kranep agar; MS=Mannitol Salt agar; S110=Staphylococcus medium No. 110.

RESULTS

Although the recoveries of S. aureus were affected by the level of inoculation, the effects were the same for each medium. At the end of the first week of incubation through the 7-month duration of the experiment, the S. aureus population in cans receiving the high and low inoculum varied between log counts of 4.7 and 8.0. The higher count was not always associated with cans receiving the high inoculum.

Regarding temperature of incubation, counts of the SED producing strain steadily decreased with time of incubation at both 30°C and room temperature. Counts of the SED producing strain increased up to 1 month of incubation then decreased only slightly at 3 and 7 months at both temperatures. Since neither of these factors affected media performance, and since there was a significant medium by strain interaction at each sampling interval (not shown), the performance of the media was determined for each strain at each sampling interval. This was done by multiple comparisons as shown in Table 1. All media performed similarly in enumerating the SED producing strain with BP and KR agars tending to yield higher but not significantly higher counts than the other two media. With the SED producing strain, S110 yielded significantly lower counts than the other media, and as the incubation period progressed, KR and BP agar tended to be favored over MS agar.

Multiple comparisons of media for 13 naturally contaminated cheese samples shown in Table 2 indicate that BP agar was significantly better than the other three media which were not markedly different from each other.

DISCUSSION

With the artificially contaminated samples, media performance was strain-dependent. The four media performed equally well in enumerating the SED producing strain which appeared to be harder than the SED producing strain in that its viability did not decline over the 7-month incubation period. In contrast, KR and BP agars proved to be significantly better than S110 agar and slightly better than MS agar in enumerating the less hardy SED producer; the viability of this organism steadily declined over the duration of the experiment. In the corned beef samples, there were few if any competing bacteria at the dilution plated and essentially all colonies picked for confirmation from any of the four media proved to thermostable nuclease positive and gave 3 to 4+ coagulase reactions (8). Conversely, competitive bacteria present in the naturally contaminated cheese samples were evident on all media at the appropriately plated dilutions. On BP agar, the percentage of presumptive colonies confirmed as S. aureus was consistently higher than those picked from the other media where selectivity and colony differentiation were less pronounced. This probably accounted for the significantly better performance of BP agar over the other three media. Similar observations were made in a previous study (9).

The results indicate that BP, KR and to a lesser extent MS are suitable for enumerating S. aureus in foods where competing bacteria are few relative to the number of S. aureus in the samples. However, BP agar is the preferred medium for samples in which the S. aureus population is likely to be obscured by competing microflora.
REFERENCES


Rayman et al., con’t. from p. 88

REFERENCES


