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Medium for the Selective Enumeration of Lactic Acid Bacteria from Foods

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A nitrite actidione polymyxin agar was developed for the enumeration of lactic acid bacteria. It was effective in recovering organisms from pure cultures and from foods.

In addition to their beneficial effects in the manufacture of fermented foods, lactic acid bacteria can also be a principal component of the microbial association of spoiled vacuum-packaged meats and meat products (1). Although various media are available for enumeration of individual genera within the lactic acid bacteria (6), no single selective medium will satisfactorily recover all members of the group. Consequently, when estimates of the total number of lactic acid bacteria in foods are required, there is a tendency to use media selective for *Lactobacillus* spp. Thus, agars containing acetate as the selective agent (3, 5) are often used. However, in cases where streptococci or certain strains of streptobacteria, or both, comprise a major portion of the flora, acetate agar could yield underestimates of the actual levels of lactic acid bacteria present (4). It was felt, therefore, that a need existed, particularly in bacteriological quality control, for a medium which would give improved recovery of the group as a whole.

The following were screened for their ability to act as selective agents: antibiotics (kanamycin and novobiocin), heme-enzyme inhibitors (azide and cyanide), hydrogen peroxide, and organic acids (lactic and sorbic). They were tested singly and in various combinations, without success. In the search for a suitable compound, it was noted that Ingram (2) had pointed out that lactic acid bacteria in general are more tolerant than most other bacteria to the inhibitory effects of nitrite, or more correctly, nitrous acid. To our knowledge, this property has never been exploited in a selective medium. A nitrite agar was therefore devised and evaluated. The basal medium contained (in grams per liter): peptone (Evans), 10.0; peptonized milk (Difco), 10.0; yeast extract (Difco), 10.0; glucose, 7.5; beef extract (Difco), 2.5; $MgSO_4 \cdot 7H_2O$, 0.575; $MnSO_4 \cdot 4H_2O$, 0.05; Tween 80, 1.0; agar, 15.0, in distilled water (pH 5.5). The ingredients were dissolved by heating, and after tempering to approximately 50 C, the pH was carefully adjusted to 5.5 (±0.05) and the volume made up to 970 ml. The medium was distributed in 97-ml amounts and autoclaved (15 lbs/15 min). Prior to use, 1.0 ml of each of (i) freshly prepared, filter-sterilized sodium nitrite solution (6.0% wt/vol), (ii) actidione solution (0.1% wt/vol) and (iii) polymyxin B solution (0.03% wt/vol) were added to each 97 ml of basal medium.

The recovery of pure cultures of *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus* and various non-lactic acid bacteria was compared on nitrite actidione polymyxin agar (NAP agar) and on basal medium. Cultures grown in APT broth (Difco) or brain-heart infusion broth (Difco) for 24 to 48 h at 30 C were serially diluted in 0.1% peptone water. A pour-plate method was used for counting. Inoculated NAP agar plates were overlaid with NAP agar and incubated at 30 C for 3 to 6 days prior to counting.

The following lactic acid bacteria yielded essentially the same counts on basal medium and NAP agar: *Lactobacillus* (18 strains), *Streptococcus* (9 strains), *Pediococcus* (3 strains), and *Leuconostoc* (1 strain). The exception was one of two strains of *Lactobacillus viridescens*, a species known to grow poorly on selective media (6).

The following were completely inhibited on NAP agar: *Salmonella* (six serotypes), *Micrococcus* (four strains), *Bacillus* (three strains), *Staphylococcus* (three strains), *Pseudomonas* (two strains), *Saccharomyces* (two strains), and one strain each of *Arizona*, *Citrobacter*, *Edwardsiella*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Microbacterium*, *Proteus*, *Providencia*, *Serratia*, and *Shigella*. Of the other non-lactic acid bacteria tested, only *Bacillus pantothenticus* was unaffected on NAP

agar. *Staphylococcus epidermidis* T-140 and *epidermidis* T-199 and *Bacillus subtilis* 9053 grew to a small extent.

NAP agar was then used to enumerate lactic acid bacteria from a variety of foods: meat and meat products (21 samples), dairy products (14 samples), and miscellaneous (4 samples). Total counts determined on both APT agar (Difco) and basal medium showed no significant difference.

With 32 out of 39 samples, the counts obtained on NAP agar compared favorably with the total catalase-negative count (determined from the proportion of catalase-negative colonies on basal medium). With the remaining seven samples (six dairy products and one sample of sourdough), NAP agar failed to recover maximum populations.

Counts on Rogosa SL (Difco) medium were also determined. In no instance did the count exceed that obtained on NAP agar. Furthermore, with certain foods (e.g., bologna, buttermilk, and ham) Rogosa SL was unsatisfactory, presumably because these samples harbored large numbers of streptococci or other lactic acid bacteria unable to grow on this medium.

We conclude from these preliminary studies that NAP agar is a suitable medium for estimating lactic acid bacteria in foods and possibly other environments.

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