New Medium for the Isolation and Enumeration of Pseudomonads

MYRON SOLBERG, VIRGINIA S. O'LEARY, AND WILLIAM E. RIHA, JR.

Department of Food Science, Rutgers University, The State University of New Jersey, New Brunswick, New Jersey 08903

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A new medium containing 200 ppb of 2-hydroxy-2',4,4'-trichlorodiphenyloxide (CH3565) and 10 ppm of cetyl-trimethyl-ammonium bromide (Cetrimide) in tryptic soy agar was developed and tested with 19 pure cultures of Pseudomonas, 20 microorganisms of other genera, commercially prepared ground beef, and laboratory-prepared inoculated ground beef. The new medium, CETCH agar, was compared with an antibiotic-containing medium. CETCH agar provided greater pseudomonad recoveries, a shorter incubation period prior to plate counting, and greater ease of preparation.

Microorganisms of the genus Pseudomonas are often responsible for food spoilage during refrigerated storage (4, 6, 7, 11, 13, 15). Pseudomonads are the predominant microorganisms present on fresh meat after several days of refrigerated storage (4, 7, 8). The proteolytic and lipolytic activity of pseudomonads produce undesirable flavors, colors, and odors in foods (11). A selective medium for the isolation and enumeration of pseudomonads would be valuable for quality assessment of fresh meat and other foods.

Masurovsky et al. (10) described a differential medium for the selection and enumeration of pseudomonads based upon the use of erythromycin and chloramphenicol as inhibitors in a basal salts solution containing L-arginine, yeast extract, and Ionagar No. 2. The preparation of the salts medium was time-consuming, as was the membrane-filter sterilization of the antibiotics. The salts tended to precipitate from the solution when it was cooled, and the precipitate interfered with colony recognition since the colonies, even after 72 hr of incubation, were small.

The need for an improved medium existed. Collins (2) suggested the possibility that 1% Cetrimide (cetyl-trimethyl-ammonium bromide) in nutrient agar might be used as a selective medium for the isolation of pseudomonads. This suggestion was predicated upon the studies which demonstrated the utility of Cetrimide for increasing pigment production in P. aeruginosa (1, 10) and for the selective isolation of P. aeruginosa (9). Trade literature indicated that CH3565 (2-hydroxy-2',4,4'-trichlorodiphenyloxide) was inhibitory to a broad spectrum of microorganisms at concentrations of less than 10 ppm, but that it did not inhibit P. aeruginosa at concentrations as high as 300 ppm.

This study was undertaken to develop a new isolation and enumeration medium for pseudomonads based upon the selectivity of Cetrimide and CH3565, and to compare the new medium with the medium of Masurovsky et al. (10).

MATERIALS AND METHODS

Culture maintenance and preparation. Stock cultures were maintained on tryptic soy (TS) agar (Difco) slants at 5 C. Broth cultures were prepared by inoculating tubes containing 10 ml of TS broth (Difco) which were grown out at least twice for 48 hr at 23 ± 1 C prior to use.

Microorganisms. The microorganisms used included 19 pseudomonads and 20 non-pseudomonads from 16 different genera. The complete list of microorganisms can be seen in Table 1.

Preparation of media. TS agar (Difco) was used as the control growth medium to support uninhibited growth.

Masurovsky et al. (MAS) agar was prepared by the method described in the literature (10).

Cetrimide (CET) agar was prepared by adding necessary quantities of a 5% aqueous solution of Cetrimide (K & K Laboratories, Inc., Plainview, N.Y.) to nutrient agar (Difco) to provide the desired final concentrations. The Cetrimide solution was sterilized by passage through a 0.22-μm membrane filter.
(Millipore Corp., Bedford, Mass.) and was added to autoclave-sterilized nutrient agar after it had cooled to 45 C.

CH3565 (CH) agar was prepared by adding necessary quantities of a 0.1% Ethyl Cellulose (ethylene glycol monoethyl ether) solution of CH3565 (Geigy Industrial Chemical Division, Ardsley, N.Y.) to TS agar to provide the desired final concentrations. The CH3565 was added to the TS agar prior to autoclave-sterilization because it is stable up to 280 to 290 C. (Bacteriostats CH3565, Geigy 1967).

Cetrimide plus CH3565 agar was prepared by adding 0.2 ml of the previously described 0.1% CH3565 solution to 1 liter of TS agar and the necessary quantities of the previously described 5% solution of Cetrimide to provide the desired final concentrations.

CETCH agar was prepared by adding 0.2 ml of 0.1% CH3565 dissolved in Ethyl Cellulose to TS agar, sterilizing the mixture at 121 C for 15 min, cooling to 45 to 48 C, and adding 0.2 ml of filter-sterilized 5% Cetrimide dissolved in water.

Recovery and selectivity tests. TS broth cultures (48 hr) of various microorganisms were serially diluted in 0.1% peptone-water. Pure cultures were pour-plated in duplicate on the various media. For meat recovery studies, cultures were spread-plated in duplicate on prepped and slightly dried plates of the various media. The plates were incubated for 2 to 3 days at 23 ± 2 C prior to counting.

Recovery of pseudomonads from commercial ground beef. Locally purchased ground beef was sampled on the day of purchase and again after 5 days of storage at 5 ± 2 C. A 50-g amount of ground beef was placed in a sterile blender cup; 450 ml of sterile 0.1% peptone-water was added, and the mixture was blended at high speed for 2 min. Serial dilutions were prepared in 0.1% peptone-water and were spread-plated on the various media. Plates were incubated at 23 ± 2 C for 2 to 3 days prior to counting.

Colonies on plates containing 20 to 200 colonies were grouped on the basis of colony morphology. Representative colonies were picked, and TS broth tube cultures were prepared. For each 10 or fewer similar-appearing colonies on a plate, one colony was subcultured up to a maximum of eight colonies representing a single group. The subcultures were grown out at 23 ± 1 C for 48 hr and then classified by the determinative scheme of Shewan et al. (14) as Pseudomonas or non-Pseudomonas.

Recovery of microorganisms from inoculated laboratory-prepared ground beef. A top round of beef (5.4 to 6.4 kg) was locally purchased. The surface was swabbed with 95% ethanol and trimmed off with sterile knives. Cores of meat were then aseptically removed with a circular knife and ground through a 0.25-inch (0.64-cm) plate in a sterile meat grinder. After thorough mixing, a control sample was removed and was subjected to analysis as previously described for commercially prepared ground beef. A mixed culture containing Citrobacter freundii, Enterobacter aerogenes, Proteus vulgaris, Alcaligenes faecalis, Micrococcus roseus, Streptococcus faecalis, Pseudomonas sp. ATCC 10838, and P. aeruginosa was prepared. Cell concentration was determined by direct microscopic count in a Petroff-Hausser counting chamber, and 5.0 × 10⁴ cells of each microorganism were added to a 400-g sample of meat by transfer of 1.0 ml or less of an appropriate 0.1% peptone-water dilution. The final concentration of added microorganisms was approximately 10⁴ cells/g. The inoculated ground beef was sampled immediately after inoculation and again after 5 days of storage at 5 ± 2 C. Sampling procedures were the same as those previously described for commercially prepared ground beef.

Data presentation. Since a TS agar control was a part of every experiment, a normalized mean value was used to express the average result of replicate experiments. The normalized mean value was computed thus:

\[ \bar{V} = \frac{V \times TS}{n} \]

where \( \bar{V} \) is the normalized mean value, \( V \) is a test medium plate count, \( TS \) is the TS agar plate count from the same experimentation as \( V \), \( TS \) is the mean of TS agar plate counts from all experiments, and \( n \) is the number of experiments on a single test medium.

RESULTS AND DISCUSSION

The recovery and inhibition of pseudomonads and other microorganisms in pure culture studies on various selective media and on noninhibitory TS agar are compared in Table 1. MAS agar was completely inhibitory to four Pseudomonas species and less than 99.9% effective against six nonpseudomonads. Cetrimide concentrations as low as 100 ppm resulted in more than 99.9% inhibition of eight Pseudomonas strains and were at least slightly inhibitory to more than half of the Pseudomonas species tested, but in most cases there was progressively greater recovery as the concentration of Cetrimide was decreased from 750 to 100 ppm. Cetrimide at 100 ppm was not an effective inhibitor against any of the five nonpseudomonads tested, which had grown well on MAS agar. CH3565 at 200 ppb did not inhibit any of the five Pseudomonas species it was screened against and effectively inhibited (>99.9% inhibition) P. vulgaris and E. cloacae, which were slightly inhibited (<99.9% inhibition) or uninhibited on MAS or CET agar (100 ppm).

The combination of 200 ppb of CH3565 and Cetrimide at concentrations below 100 ppm indicated that 10 ppm of Cetrimide would maintain effectiveness against those nonpseudomonads which were not inhibited by 200 ppb of CH3565 (M. roseus and Sarcina lutea).

These results indicate that the suggested use
| Microorganism                        | TS   | MAS   | Cetrimide (ppm) | CH3565 (ppb) | 200 ppb of CH3565 + Cetrimide (ppm) | CETCH   |
|-------------------------------------|------|-------|-----------------|--------------|-------------------------------------|---------|
|                                     | 750  | 500   | 300  | 100   | 500   | 300   | 200  | 100   | 50 | 10 | 5   |
| Pseudomonas aeruginosa F-10063      |      |       |      |       |     |      |      |      |     |     |      | 5.6 x 10^6 |
| P. chlorophis NRRL B2075            | 8.4 x 10^6 | 6.4 x 10^6 | 4.0 x 10^7 | 1.2 x 10^9 | 8.2 x 10^9 | 4.8 x 10^9 |      |      |     |     |      | 5.9 x 10^6 |
| P. denitrificans NRRL B-1028        | 7.6 x 10^6 | <     | <    | 8    | <    |      |      |      |     |     |      | 1.2 x 10^6 |
| P. fluorescens NRRLB-10              | 6.9 x 10^6 | 6.2 x 10^6 | <    | <    | 2.3 x 10^4 | 1.4 x 10^4 |      |      |     |     |      | 1.5 x 10^7 |
| P. fragi NRRL B-25                   | 1.1 x 10^6 | 7.2 x 10^7 | <    | 1.1 x 10^4 | 1.5 x 10^4 | 2.5 x 10^7 |      |      |     |     |      | 1.8 x 10^6 |
| P. indoloxidans NRRL B-769           | 1.1 x 10^6 | 6.4 x 10^8 | <    | <    | 3.0 x 10^7 | 7.5 x 10^3 |      |      |     |     |      | 6.4 x 10^6 |
| P. oleovarans NRRL B-778             | 7.6 x 10^7 | 3.2 x 10^7 | 5.5 x 10^7 | 1.8 x 10^8 | 4.8 x 10^4 | 8.4 x 10^4 |      |      |     |     |      | 1.6 x 10^7 |
| P. ovalis NRRL B-8                   | 5.0 x 10^6 | 8.8 x 10^6 | 2.0 x 10^9 | 2.4 x 10^7 | 4.5 x 10^6 | 5.6 x 10^7 |      |      |     |     |      | 3.5 x 10^6 |
| P. ovalis RU #243                   | 1.1 x 10^6 | 1.9 x 10^7 | <    | 5.7 x 10^4 | 1.4 x 10^6 | 7.3 x 10^4 |      |      |     |     |      | 9.7 x 10^6 |
| P. perolans var. Gdansk NRRL B-1123 | 4.4 x 10^6 | 5.8 x 10^6 | <    | 8.5 x 10^4 | 1.1 x 10^6 | 4.2 x 10^7 |      |      |     |     |      | 1.6 x 10^8 |
| P. putida NRRL B-13                  | 3.4 x 10^7 | <     | <    | <    | <    |      |      |      |     |     |      | < |
| P. putrefaciens NRRL B-1449          | 2.2 x 10^7 | <     | <    | <    | <    |      |      |      |     |     |      | < |
| Pseudomonas sp. ATCC 11922           | 3.5 x 10^6 | <     | <    | <    | <    |      |      |      |     |     |      | < |
| Pseudomonas sp. ATCC 9229            | 1.7 x 10^5 | 1.3 x 10^5 | 1.1 x 10^6 | 2.6 x 10^9 | 9.1 x 10^7 | 8.5 x 10^8 |      |      |     |     |      | 1.2 x 10^9 |
| Pseudomonas sp. NRRL B-1885          | 4.1 x 10^6 | 5.4 x 10^6 | 2.1 x 10^6 | 1.4 x 10^9 | 2.1 x 10^6 | 1.3 x 10^8 |      |      |     |     |      | 8.0 x 10^9 |
| Pseudomonas sp. ATCC 10583           | 3.6 x 10^7 | 2.0 x 10^7 | 1.1 x 10^6 | 1.8 x 10^9 | 2.1 x 10^6 | 2.8 x 10^9 |      |      |     |     |      | 4.5 x 10^9 |
| P. stutzeri NRRL B-927               | 2.9 x 10^6 | 3.7 x 10^6 | <    | <    | 1.9 x 10^6 | 2.1 x 10^2 |      |      |     |     |      | 1.2 x 10^9 |
| Pseudomonas syxanthax NRRL B-780     | 1.0 x 10^6 | 7.8 x 10^6 | 1.1 x 10^6 | 2.4 x 10^4 | 4.1 x 10^6 | 3.0 x 10^7 |      |      |     |     |      | 8.3 x 10^9 |
| P. taetrolens NRRL B-14              | 1.5 x 10^6 | 1.5 x 10^6 | <    | 4.6 x 10^3 | 2.4 x 10^6 | 6.1 x 10^3 | 3.7 x 10^3 | 1.6 x 10^3 | 1.5 x 10^6 | 1.5 x 10^6 | < |
| Achromobacter xerosis ATCC 14780     | 6.8 x 10^6 | 4.0 x 10^6 | <    | <    | <    |      |      |      |     |     |      | < |
| Alcaligenes faecalis ATCC 8750       | 1.6 x 10^8 | 6.8 x 10^6 | <    | 7.2 x 10^4 | 1.1 x 10^8 | 1.5 x 10^6 | 2.1 x 10^9 | 1.6 x 10^6 | 1.5 x 10^6 | 1.3 x 10^6 | 1.5 x 10^9 | 1.4 x 10^9 |
| Bacillus megaterium ATCC 4658        | 6.8 x 10^7 | <     | <    | <    | <    |      |      |      |     |     |      | < |
| Citrobacter freundii ATCC 8990       | 5.6 x 10^5 | 4.3 x 10^4 | <    | 5.2 x 10^4 | 5.2 x 10^7 | 4.1 x 10^5 | 5.5 x 10^6 | 4.4 x 10^7 | 3.9 x 10^8 | 4.7 x 10^9 | 5.9 x 10^8 | 7.3 x 10^8 | 5.4 x 10^9 |
| Enterobacter aerogenes ATCC 13048     | 2.0 x 10^6 | 1.3 x 10^6 | <    | 1.0 x 10^3 | 1.6 x 10^6 | contam | 3.5 x 10^8 |      |      |     |      | 1.2 x 10^9 |
| E. cloacae ATCC 10347                | 3.2 x 10^6 | 3.8 x 10^6 | <    | 2.2 x 10^3 | 1.2 x 10^3 |      | 1.0 x 10^9 | 2.3 x 10^8 |      |      | 3.4 x 10^9 |
| Escherichia coli ATCC 11775          | 2.5 x 10^8 | <     | <    | <    | <    |      |      |      |     |     |      | < |
| Flavobacterium capsulatum ATCC 14666 | 3.9 x 10^5 | 1.4 x 10^3 | <    | <    | <    | <    |      |      |     |     |      | < |
| Kurthia zopfii ATCC 6900             | 3.2 x 10^6 | <     | <    | <    | <    |      |      |      |     |     |      | < |
| Leuconostoc mesenteroides ATCC 8293  | 8.0 x 10^6 | <     | <    | <    | 1.2 x 10^5 | 1.4 x 10^7 | 1.7 x 10^4 | 1.3 x 10^6 |      |     |      | 6.1 x 10^9 |
| Micrococcus roseus ATCC 516          | 1.5 x 10^6 | <     | <    | <    | <    |      |      |      |     |     |      | < |

*Response of pure cultures to various selective media in comparison to response on tryptic soy agar determined by pour plating.*
| Organism                     | Organisms (per ml) | Organisms (per ml) | Organisms (per ml) | Organisms (per ml) | Organisms (per ml) | Organisms (per ml) | Organisms (per ml) | Organisms (per ml) | Organisms (per ml) | Organisms (per ml) |
|------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Proteus vulgaris RU #133     | 5.1 x 10^5         | 3.8 x 10^7         | < 4.0 x 10^8       | <                  | <                  | <                  | <                  | <                  | <                  | 2.6 x 10^1         |
| Saccharomyces cerevisiae ATCC 834 | 1.2 x 10^7         | 2.5 x 10^7         | < 1.5 x 10^7       | 3.3 x 10^9         | < 3.8 x 10^9       | < 3.8 x 10^9       | <                  | <                  | 3.7 x 10^4         |
| Salmonella typhimurium ATCC 13311 | 4.9 x 10^8         | < 6.5 x 10^8       | < 1.1 x 10^7       | 3.3 x 10^9         | < 3.8 x 10^9       | < 3.8 x 10^9       | <                  | <                  | 1.6 x 10^4         |
| Sarcina lutea W. Iverson-62755 | 2.6 x 10^8         | < 2.3 x 10^8       | < 3.9 x 10^9       | 4.0 x 10^9         | < 8.0 x 10^9       | < 8.0 x 10^9       | <                  | <                  | <                  |
| Staphylococcus aureus ATCC 9664 | 2.1 x 10^8         | < 2.1 x 10^8       | < 9.3 x 10^9       | 8.0 x 10^9         | < 8.0 x 10^9       | < 8.0 x 10^9       | <                  | <                  | <                  |
| S. aureus ATCC 6538P         | 1.9 x 10^9         | < 1.9 x 10^9       | < 9.3 x 10^9       | 8.0 x 10^9         | < 8.0 x 10^9       | < 8.0 x 10^9       | <                  | <                  | <                  |
| Streptococcus fecalis ATCC 8043 | 1.9 x 10^9         | < 1.9 x 10^9       | < 9.3 x 10^9       | 8.0 x 10^9         | < 8.0 x 10^9       | < 8.0 x 10^9       | <                  | <                  | <                  |
| S. lactis ATCC 11454         | 7.4 x 10^8         | < 7.4 x 10^8       | < 9.3 x 10^9       | 8.0 x 10^9         | < 8.0 x 10^9       | < 8.0 x 10^9       | <                  | <                  | <                  |

* Results are expressed as recoverable organisms per milliliter of a 48-hr culture incubated at 23 ± 2°C in tryptic soy broth; < = less than 30 organisms/ml.

* TS = tryptic soy agar; MAS = differential medium of Masurovsky et al. (10); Cetrimide = cetyl-trimethyl-ammonium bromide in TS agar; CH3565 = 2 hydroxy 2',4,4'-trichlorophenoloxide in TS agar; CETCH = 200 ppb of CH3565 + 10 ppm of Cetrimide in TS agar.
of Cetrimide as the basis for a general pseudomonad isolation medium (2) is unsatisfactory. The suggested 1% level of Cetrimide (2) was based upon an impure form of the substance, as was shown by Lowbury and Collins (9), who established the refined chemical level at 0.03%. Even 0.03% Cetrimide was too inhibitory to pseudomonads to be used in an isolation and enumeration medium. These observations were similar to those reported by Goto and Enomoto (5), who reported adverse effects of Cetrimide concentrations above 0.03% on P. aeruginosa and the lack of inhibition of some gram-negative rods in the 0.03% Cetrimide medium. Curry and Butera (3) reported inhibition of pseudomonads by 50 and 100 ppm of CH3565 ranging from 66 to 99%, demonstrating the unsuitability of such high CH3565 levels in an isolation and enumeration medium. Pseudomonas isolation agar (Difco), which contains 25 ppm of CH3565, was not evaluated for recovery levels by Curry and Butera (3), but appeared very similar to medium containing 50 ppm of CH3565 with respect to isolation characteristics.

CETCH agar was completely inhibitory to three of the four pseudomonads which were inhibited by MAS agar. The overall recovery relative to TS agar of those Pseudomonas species which grew well on both media was 80% for CETCH agar and 70% for MAS agar. The colonies on CETCH agar were equivalent in size to those on TS agar and were countable after 48 hr of incubation at 23 ± 2 C, whereas the colonies on MAS agar were not countable until at least 72 hr of incubation elapsed and then were small enough to be confused with precipitated salts in the medium. The data of Table 1 were statistically analyzed by use of analysis of variance techniques. Recovery levels were not significantly different when CETCH agar was compared with MAS agar or when CETCH, MAS, and TS agars were compared with one another.

The inhibition of E. cloacae was not substantiated in CETCH agar, and was probably an artifact in the previous experiment. The inhibition of P. vulgaris was reproducible and was the only selectivity improvement over MAS agar. Alcaligenes, Enterobacter, and Citrobacter were not inhibited on either MAS or CETCH agar. A recent publication (12) indicated the utility of diamide as a selective enrichment ingredient for the isolation of pseudomonads. The results of the diamide study (12) indicate a potential to overcome some of the selectivity problems, and diamide medium should be evaluated.

The recovery of pseudomonads from commercially prepared ground beef is presented in Table 2. Nonpseudomonads were not recovered on either MAS or CETCH agar before or after sample storage. The freshly purchased samples contained an average of 20% nonpseudomonads, and the 5-day stored samples contained an average of 10% nonpseudomonads. The percent recovery of pseudomonads was higher on CETCH agar than on MAS agar for every sample. Analysis of variance demonstrated that recoveries on CETCH and MAS agars were significantly different (P < 0.05), whereas there was no significant difference between CETCH and TS agars (P < 0.01).

The common food-spoilage and food-poisoning microorganisms which would be found in fresh meat, such as Bacillus, Streptococcus, Micrococcus, Flavobacterium, Achromobacter, Kurthia, Escherichia, Salmonella, and Staphylococcus, were inhibited by both MAS and CETCH agars, as is evident in the pure culture studies in Table 1.

The recovery of pseudomonads and the selectivity of MAS and CETCH agars when challenged by a mixed culture containing large numbers of noninhibited microorganisms in ground beef is presented in Table 3. The failure to obtain ground meat of very low microbial contamination through aseptic preparation techniques is evident in samples 1 and 2. Samples 3 and 4 initially contained less than 2 × 10⁴ microorganisms/g of meat. The results in Table 3 again show statistically significant (P < 0.05) higher recovery levels on CETCH agar than on MAS agar. Inoculated unstored meat samples 3 and 4 contained a somewhat lower inoculum level than was anticipated. This may have been the result of nonviable cells which were counted by the direct microscopic counting technique, or it may have resulted from cold shock or other cell damage occurring during transfer and mixing into the chilled ground meat system.

Table 3 also illustrates that both MAS and CETCH agars will permit the growth of some resistant nonpseudomonad microorganisms in a meat sample. In this study, selected resistant microorganisms were inoculated into the meat and were recovered in some experiments both immediately after inoculation and after refrigerated storage for 5 days. The differences between Pseudomonas count and total count on the selective media are the result of the resistant nonpseudomonad microorganisms. The very low dilution samples, in which considerable quantities of meat were present (10⁻¹ dilution), generally developed spreading
Table 2. Recovery of pseudomonads and other microorganisms from commercially prepared ground beef on selective and nonselective media

| Storage time (days) | Sample no. | TS agar* | CETCH agar* | MAS agar* |
|---------------------|------------|-----------|-------------|-----------|
|                     |            | Total plate count | Total Pseudomonas count | Percent Pseudomonas | Pseudomonas recovery (%) | Pseudomonas plate count | Pseudomonas recovery (%) |
| 0                   | 1          | 1.6 x 10^4 | 1.1 x 10^4 | 70 | 4.1 x 10^4 | 40 | 1.8 x 10^4 | 20 |
|                     | 2          | 6.7 x 10^4 | 3.7 x 10^3 | 60 | 3.8 x 10^4 | 100 | 2.2 x 10^4 | 60 |
|                     | 3          | 2.3 x 10^4 | 2.1 x 10^4 | 90 | 2.3 x 10^4 | 100 | 1.1 x 10^4 | 5  |
| 5                   | 1          | 3.5 x 10^4 | 3.4 x 10^4 | 100 | 2.4 x 10^4 | 70  | 6.0 x 10^4 | 2  |
|                     | 2          | 3.7 x 10^4 | 3.6 x 10^4 | 100 | 1.9 x 10^4 | 50  | 3.5 x 10^4 | 10 |
|                     | 3          | 2.9 x 10^4 | 2.9 x 10^4 | 80  | 2.5 x 10^4 | 90  | 3.4 x 10^4 | 10 |
|                     | 4          | 7.8 x 10^4 | 7.8 x 10^4 | 70  | 1.1 x 10^4 | 400 | 1.3 x 10^4 | 40 |

*All counts are expressed in organisms per gram.

Table 3. Recovery of pseudomonads and other microorganisms from aseptically removed cores of bovine semimebranous muscle ground and inoculated with a mixed culture of microorganisms consisting of meat-spoilage and selective medium challenging genera

| Storage time (days) | Sample no. | Treatment | TS agar* | CETCH agar* | MAS agar* |
|---------------------|------------|-----------|-----------|-------------|-----------|
|                     |            | Total plate count | Total Pseudomonas count* | Percent Pseudomonas | Pseudomonas plate count | Percent Pseudomonas recovered* | Pseudomonas plate count | Percent Pseudomonas recovered* |
| 0                   | 1          | Prior to inoculation | 9.8 x 10^4 | 8.8 x 10^4 | 90 | 9.7 x 10^4 | 100 | 2.4 x 10^4 | 30 |
|                     | 2          | Contaminated | 1.2 x 10^5 | 1.1 x 10^4 | 90 | 1.5 x 10^4 | 100 | 3.9 x 10^4 | 40 |
|                     | 3          | <            | <            | < | < | < | < | < |
| 0                   | 1          | Inoculated | 1.9 x 10^4 | 1.9 x 10^4 | 100 | 7.2 x 10^4 | 40 | 1.8 x 10^4 | 10 |
|                     | 2          | Inoculated | 1.7 x 10^5 | 1.7 x 10^4 | 100 | 6.9 x 10^4 | 40 | 1.3 x 10^4 | 8 |
|                     | 3          | Inoculated | 3.5 x 10^3 | 2.6 x 10^3 | 50  | 1.2 x 10^4 | 50  | < | < |
|                     | 4          | Inoculated | 4.7 x 10^3 | 2.8 x 10^3 | 60  | 2.2 x 10^4 | 80  | < | < |
| 5                   | 1          | Inoculated | 3.5 x 10^10 | 3.5 x 10^10 | 100 | 3.1 x 10^10 | 90 | 1.2 x 10^10 | 30 |
|                     | 2          | Inoculated | 3.6 x 10^10 | 2.1 x 10^10 | 60  | 2.5 x 10^10 | 100 | 2.5 x 10^10 | 10 |
|                     | 3          | Inoculated | 1.7 x 10^7 | 1.7 x 10^7 | 100 | 1.6 x 10^7 | 90  | 3.5 x 10^6 | 2  |
|                     | 4          | Inoculated | 3.0 x 10^7 | 3.0 x 10^7 | 100 | 2.0 x 10^7 | 70  | 3.4 x 10^6 | 10 |
colonies typical of *Proteus* on both MAS and CETCH agars, thus making these plates uncountable. CH3565, which was controlling the *Proteus* growth, is rendered ineffective at low concentrations by blood serum (Bacteriostat CH3565, Geigy Industrial Chemicals Division, 1967). The presence of significant quantities of meat and meat extract may yield a similar response which may then be overcome by further dilution.

When only those recovery values of 100% or less were used in the computation, the overall recovery of pseudomonads from the meat samples on CETCH agar was 80%, which is equivalent to the recovery from the pure culture studies. Only 20% of the pseudomonads were recoverable from meat on MAS agar, compared to 70% which were recoverable in the pure culture studies. Gardner (4) reported 70% recovery of a pure *P. fluorescens* culture on MAS agar but did not determine the percent recovery from an inoculated meat sample. There is a remote possibility that cells subjected to refrigeration temperatures in fresh meat at pH 5.3 to 5.6 will develop metabolic lesions which are more evident under the challenge of MAS agar than on CETCH agar, because MAS agar is a minimal growth-supporting medium.

CETCH agar seems to be a suitable isolation and enumeration medium for *Pseudomonas* species in food products which do not have a significant number of *Alcaligenes*, *Enterobacter*, or *Citrobacter* species as a major component of their natural microflora. CETCH agar is more easily prepared than MAS agar, as it requires the addition of only two substances, one of which is heat-stable, to commercially available TS agar, rather than the preparation of a 10-component medium in addition to two heat-labile antibiotic solutions. CETCH agar supports the growth of full-sized colonies of normal morphology in 48 hr at 23 ± 2°C rather than the small abnormal colonies which are not countable before 72 hr of incubation on MAS agar. CETCH agar permits approximately 40 to 100% recovery of pseudomonads compared to recoveries of 5 to 50% on MAS agar when ground beef is the test substrate.

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