

TOOLS FOR MICROBIAL DETECTION

COMPARISON OF DRY SHEET MEDIA AND CONVENTIONAL AGAR MEDIA METHODS FOR ENUMERATING YEASTS AND MOLDS IN FOOD

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Several dry sheet media and conventional agar media are commercially available for enumerating microorganisms in foods. The most extensively used dry sheet method is the 3M Petrifilm system which consists of a card coated with one of several dry media containing nutrients, a cold-water-soluble gel, and 2,3,5-triphenyl tetrazolium chloride (TTC) to facilitate visualization of colonies. A plastic film attached to one end of the card protects the medium before and after depositing the test sample. The Nissui Compact Dry system consists of a modified plastic Petri plate containing a self-diffusible dry medium and a chromogenic enzyme substrate (5-bromo-4-chloro-3-indoxyl phosphate, *p*-toluidine salt) to facilitate counting. After depositing the sample on the medium, a cap is applied. Both systems offer conveniences not associated with conventional agar media. Dichloran 18% glycerol (DG18) agar is used in some laboratories as a general purpose medium to enumerate yeasts and molds in foods. This medium was formulated to favor recovery of xerophiles from low-moisture and intermediate-moisture foods but has been reported to also perform favorably with dichloran rose Bengal chloramphenicol (DRBC) agar for recovering yeasts and molds with high a_w . Exceptions are dried foodstuffs containing slow-growing xerophiles. A side-by-side comparison of the performance of 3M Petrifilm YM (yeast and mold) count plates, Compact Dry YM plates, DRBC agar, and DG18 agar for enumerating yeasts and molds naturally occurring in foods has not been reported. We undertook a study to evaluate these methods for enumerating yeasts and molds in a wide range of raw and processed foods of animal and plant origin.

We compared Nissui Compact Dry Yeast and Mold plates (CDYM), 3M Petrifilm Yeast and Mold count plates (PYM), DRBC agar, and DG18 agar for enumerating yeasts and molds naturally occurring in 97 foods (grains, legumes, raw fruits and vegetables, nuts, dairy products, meats, and miscellaneous processed foods and dry mixes). Correlation coefficients for plates incubated for 5 days were: DG18 vs DRBC (0.93), PYM vs DRBC (0.81), CDYM vs DG18 (0.81), PYM vs DG18 (0.80), CDYM vs DRBC (0.79), and CDYM vs PYM (0.75). The number of yeasts and molds recovered from a group of foods ($n = 32$) analyzed on a weight basis (CFU/g) was not significantly different ($\alpha = 0.05$) when samples were plated on DRBC, DG18, PYM, and CDYM. However, the order of recovery from foods ($n = 65$) in a group analyzed on a unit or piece basis, or a composite of both groups ($n = 97$), was DRBC > DG18 = CDYM > PYM. Compared to PYM, CDYM recovered equivalent, significantly higher ($\alpha = 0.05$), or significantly lower ($\alpha = 0.05$) numbers of yeasts and molds in 51.5, 27.8, and 20.6%, respectively, of the 97 foods tested; respective values were 68.8, 15.6, and 15.6% in the small group ($n = 32$) and 43.1, 33.8, and 23.1% in the large group ($n = 65$) of foods. The two groups contained different types of foods, the latter consisting largely (73.8%) of raw fruits ($n = 16$) and vegetables ($n = 32$). Differences in efficacy of the four methods in recovering yeasts and molds from foods in the two groups are attributed in part to differences in genera and predominant mycoflora. While DG18 agar, CDYM, and PYM appear to be acceptable for enumerating yeasts and molds in the foods analyzed in this study, overall, DRBC agar recovered higher numbers from the 97 test foods, thereby supporting its recommended use as a general purpose medium for mycological analysis.

SURFACE-STERILIZATION METHODS FOR *ESCHERICHIA COLI* O157:H7 ON LETTUCE (*LACTUCA SATIVA* L.)

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Many outbreaks of *Salmonella* and *Escherichia coli* O157:H7 infections have been associated with consumption of fresh-cut leafy greens in the past decade. Questions remain regarding the ability of these pathogens to become internalized within lettuce and spinach. To differentiate internalized populations from surface contamination, an effective surface-sterilization method for lettuce is needed and was the focus of this study.

Iceberg lettuce (*Lactuca sativa* L.) was purchased from a local grocery store and cut into 3 x 3 cm pieces. Lettuce roots were purchased from a local farmer. Leaf pieces and roots were inoculated by immersing in 10^8 CFU of a five-strain mixture of GFP-labeled *E. coli* O157:H7/ml for 10 min at room temperature. Inoculated

samples were put in a laminar flow biosafety cabinet for 30 min before further treatment. Thirteen surface-sterilization methods (including sodium hypochlorite, ethanol, HgCl₂, and hydrogen peroxide) were compared for their efficacy in killing/removing *E. coli* O157:H7 on lettuce leaf and root surfaces. Treated samples were washed 5 times with sterile water, and then assayed for *E. coli* O157:H7.

Among the 13 surface-sterilization methods evaluated, *E. coli* O157:H7 was not detected by enumeration with a direct plating procedure on treated samples for 3 treatments, including 20 min with 10,000 ppm sodium hypochlorite and 2 treatments containing ethanol and HgCl₂. There were 2.8 to 4.4 CFU *E. coli* O157:H7/leaf piece or root after surface-sterilization for the other methods. Plant tissue prints on agar and enrichment culture results were consistent with enumeration results. Our overall data revealed that the best surface-sterilization method for lettuce leaves and roots was dipping in 80% ethanol for 10 s, followed by immersion in 0.1% HgCl₂ for 10 min.

ISOLATION OF *YERSINIA PESTIS* FROM FOOD USING IMMUNOMAGNETIC BEADS COATED WITH MONOCLONAL ANTIBODY 6B5

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Yersinia pestis is notoriously known for causing the plague. Most immunoassays available for detecting *Y. pestis* in humans or environmental samples are based on recognition of the organism's fraction-1 antigen (F-1). Studies have revealed that the production of F-1 is temperature-sensitive and is produced at 37°C, but not at temperatures less than 26°C. *Y. pestis*, when present in water, fruit juice, milk, food and the environment, may not produce sufficient F-1 for detection because of this temperature dependence of F-1 production. Hence, currently available immunological methods based on detection of *Y. pestis* F-1 would not likely detect *Y. pestis* directly without pre-culturing samples at 37°C. We have previously reported a non-F-1-specific monoclonal antibody (MAb 6B5) that was developed for detection of *Y. pestis*. The objective of this study was to test and compare the ability of magnetic beads coated with MAb 6B5 or polyclonal antibodies against *Y. pestis* to isolate *Y. pestis* from foods and water.

Water and food samples (milk, ground beef, ground chicken and ground pork) were spiked with *Y. pestis* at cell numbers of 0, 1, 10, and 100 CFU/ml or g. Magnetic beads, which were coated with either purified polyclonal (rabbit) antibody against *Y. pestis* or MAb 6B5, were used for the isolation of *Y. pestis* in the food samples by using a Pathatrix cell concentration apparatus. Two enrichment times (6 and 24 h) in brain heart infusion broth at 37°C were evaluated. All presumptive colonies of *Y. pestis* were confirmed by biochemical and PCR assays. Results revealed MAb 6B5-coated magnetic beads were similar in concentrating *Y. pestis* for detection as magnetic beads coated with polyclonal antibodies, especially when samples were enriched for 6 h. However, the selectivity for *Y. pestis* on the magnetic beads coated with MAb 6B5 was greater than those coated with polyclonal antibodies. There were about 10 times less bacterial colonies on *Yersinia* selective agar plates from MAb 6B5-coated magnetic beads than from polyclonal antibody-coated beads. Furthermore, ca. 30% of the colonies from MAb 6B5-coated beads were *Y. pestis*, whereas only ca. 5% of colonies from polyclonal antibody-coated beads were *Y. pestis*. The magnetic beads coated with MAb 6B5 more selectively isolated *Y. pestis* from foods and water than polyclonal antibody-coated beads.

SILVER NANOROD ARRAY AS A SERS SUBSTRATE FOR FOODBORNE PATHOGENIC BACTERIA DETECTION

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Surface enhanced Raman scattering (SERS) using novel silver nanorod array substrates has been used for the detection of pathogenic bacteria. The substrate consists of a base layer of 500 nm silver film on a glass slide and a layer of silver nanorod array with length of ~1 μm produced by oblique angle deposition method at a vapor incident angle of 86°. Spectra from whole cell bacteria, Generic *Escherichia coli*, *Escherichia coli* O157:H7, *E. coli* DH 5α, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Salmonella typhimurium*, and bacteria mixtures, have been obtained. This SERS active substrate can detect spectral differences between Gram types, different species, their mixture, and strains. Principle component analysis method has been applied to classify the spectra. Viable and nonviable cells have also been examined and significantly reduced SERS responses at major Raman bands were observed for nonviable cells. The SERS spectra of bacteria on single cell level excited at low incident laser power (12 μW) and short collection time (10 s) has also been demonstrated. These results indicate that the SERS-active silver nanorod arrays substrate is a potential analytical sensor for rapid identification of microorganisms with a minimum sample preparation procedure.