TOOLS FOR MICROBIAL DETECTION

EVALUATION OF DIRECT PLATING METHODS TO ENUMERATE *ALICYCLOBACILLUS* IN BEVERAGES

(M. B. Murray, J. B. Gurtler, J.-H. Ryu, M. A. Harrison, and L. R. Beuchat)

Spores of *Alicyclobacillus* species are known to survive heat pasteurization processes applied to fruit, vegetable, and fruit/vegetable-based beverages, fruit concentrates and purees, sugar, sugar syrups, tea, isotonic drinks (sports drinks), and other low-pH products. The development of a standard direct plating method to enumerate *Alicyclobacillus* has been difficult because of different pH and temperature optima for growth of some strains of various *Alicyclobacillus* species. Several agar media have been formulated to detect specific species but eventually used to enumerate *Alicyclobacillus* species in general. In addition to differences in nutrient content in enumeration media, the pH ranges from 3.5 to 5.6. The incubation temperature used in various laboratories is 40 - 55ºC and the incubation time is 1 - 7 days. Recommended culture media, incubation temperature, and incubation time differ among countries and groups representing the juice and beverage industry in various countries and geographic regions. We did a study to evaluate ten agar media for their suitability to support spore germination and colony development by six strains of *Alicyclobacillus acidoterrestris*, three strains of *Alicyclobacillus acidocaldarius*, and one strain of *Alicyclobacillus cycloheptanicus*. The influence of plating method (pour versus spread), incubation temperature (43°C and 50°C), and incubation time (up to 10 days) on colony development were determined. K agar, *Alicyclobacillus* medium (ALI agar), and *Bacillus acidoterrestris* thermophilic (BAT) agar recovered the highest numbers of spores. Orange serum agar and Hiraishi glucose yeast extract agar were the least suitable. Overall, surface plating was superior to pour plating and, with the exception of one strain of *A. acidocaldarius* which grew better at 50°C, incubation of K agar, ALI agar, and BAT agar plates at 43°C or 50°C resulted in recovery of equivalent numbers of spores. Essentially all viable spores were detected on media incubated for 3 days at 43°C. The ability of one strain of each *Alicyclobacillus* species to grow in ten non-carbonated commercially manufactured beverages at 30°C and 43°C was markedly affected by the composition of the beverages. Results show that surface plating samples on BAT agar, followed by incubating plates at 43°C for 3 days provide the most suitable conditions to enumerate ten strains of three species of *Alicyclobacillus* most commonly responsible for spoilage of beverages.

CRYOTOLERANCE, ATTACHMENT, AND RECOVERABILITY OF *ESCHERICHIA COLI* O157:H7 AND SELECTED SURROGATES FROM ROMAINE LETTUCE LEAF SURFACES

(M.A. Harrison)

A non-pathogenic bacterial species that responds to food processing treatments in a manner equivalent to a foodborne pathogen can potentially be used in actual food processing facilities to evaluate the effectiveness of the process to remove or eliminate the pathogen. Using the surrogate reduces the concerns related to the intentional introduction of pathogens in the food processing environment. It is important that the surrogate behave in a manner similar to that of the pathogenic microorganisms of interest. Surrogates have been evaluated and recommended for some heat, acidification, and drying procedures used in food processing. There is little work related to having suitable surrogates available to evaluate the fate of pathogens on refrigerated foods. With recent foodborne illness outbreaks related to fresh produce, it is important to have tools which would allow for the evaluation of possible microbial intervention processes on these refrigerated products. The objectives of the study were to determine if non-pathogenic *E. coli* strains could serve as surrogates of *E. coli* O157:H7 for attachment and recoverability studies involving chilled produce and to investigate the effect starvation and cold stress have on the behavior of *E. coli* O157:H7 and selected surrogates.

Five nonpathogenic *E. coli* strains were evaluated for behavior similar to that of *E. coli* O157:H7. The organisms were grown under conditions with minimal nutrients to create starved conditions. To evaluate response to cryotolerance, starved cells were frozen in sterile deionized water at –18°C for 1, 2, 4 and 7 d. After storage at –18°C, control and starved cells were thawed at room temperature and the viable population was determined. To determine whether the possible surrogates attach to lettuce surfaces and can be recovered or removed from the surfaces at a similar rate to *E. coli* O157:H7, romaine lettuce pieces were inoculated with each organism. After 1 hour, pieces were gently rinsed with either sterile deionized water or with chlorinated water.

All *E. coli* strains tested exhibited cryotolerance with less than 1 log CFU/ml decrease over 7 days of storage. In determining the attachment rate to lettuce, it was determined that *E. coli* ATCC 25922 exhibited the greatest

attachment rate (79% compared to *E. coli* O157:H7). After chlorine treatment, *E. coli* ATCC 25922 population decreased by a similar rate to that of *E. coli* O157:H7. *E. coli* ATCC 25922 also had similar hydrophobicity compared to *E. coli* O157:H7. Cryotolerance and survival of starved organisms were measured after *E. coli* ATCC 25922 and *E. coli* O157:H7 were held in sterile deionized water for starvation (37°C for 4 hours, 20°C for 24 hours, or 4°C for 7 days). Both stressed *E. coli* O157:H7 and stressed *E. coli* ATCC 25922 exhibited greater cryotolerance than nonstressed control cells. Populations of *E. coli* ATCC 25922 and *E. coli* O157:H7 were reduced by similar amounts (by approx. 99%) after washing with chlorinated water regardless of starvation conditions. *E. coli* ATCC 25922 was found to be a useful surrogate for *E. coli* O157:H7 for studies involving attachment and recoverability of chilled produce.

SILVER NANOROD ARRAY AS A SERS SUBSTRATE FOR *ESCHERICHIA COLI* O157:H7 DETECTION

(H.Y. Chu, Y. Liu, Y. Huang, and Y. Zhao)

The ability to identify pathogens rapidly, nondestructively and distinctively has major benefit to epidemic outbreak and bioterrorism prevention. Surface-enhanced Raman spectroscopy (SERS) has been used as an analytical tool to observe trace amount of chemical and biological molecules due to its capability of giving real-time molecular vibrational information under ambient conditions. As an attempt to meet these needs, we have integrated the silver nanorod arrays substrate fabricated by oblique angle deposition (OAD) technique into a fiber optic surface-enhanced Raman spectroscopy as a portable pathogen sensor for on-site inspection. The substrate consists of a base layer of 500 nm silver film first deposited onto a glass slide and a layer of silver nanorod array with length of ~1 μm deposited by OAD method at a vapor incident angle of 86°. The portable sensor has a sensitivity of 10^{-14}. Moles for trans-1,2-bis(4-pyridyl)ethene (BPE). The SERS spectra for *E. coli* O157:H7 and generic *E. coli* were obtained and compared, and distinct spectroscopic fingerprints (Raman peaks around the 735cm $^{-1}$, 1030 cm^{-1}, 1330 cm^{-1}, 1450 cm^{-1} band) for *E. coli* O157:H7 have been observed. Those SERS spectra and peaks from *E. coli* O157:H7 were reproducible among different batch of substrates and bacteria samples. This study shows that the integrated OAD silver nanorod arrays substrates and fiber Raman system is a potential portable pathogen sensor for on-site food inspection.

A MONOCLONAL ANTIBODY THAT BINDS SURFACE NON-FRACTION 1 ANTIGENS OF *YERSINIA PESTIS*

(T. Zhao, P. Zhao, M.P. Doyle, D. Gottfried, and J. Xu)

Most available diagnostic approaches for *Yersinia pestis* are based on the detection of fraction-1 antigen (F1), which is the dominant surface antigen expressed by the pathogen when it is grown at 37°C. A monoclonal antibody was developed based on the detection of non-F-1 surface antigen. F-1 deficient *Y. pestis* cells were induced by growing cultures in brain heart infusion broth adjusted to pH 5.5 with lactic acid and at 15°C for 7 days with 30 continuous weekly transfers under the same growth conditions. The loss of expression of F-1 by these cells was confirmed by an immunoblot of OMPs using a monoclonal antibody that specifically recognizes F-1. The F-1-deficient *Y. pestis* cells were used to immunize BALB/c mice from which a monoclonal antibody (IgG1) that specifically recognizes *Y. pestis,* with or without F-1, was obtained. This monoclonal antibody (6B5) did not cross-react with enteric bacteria, including *Y. enterocolitica, Salmonella* Typhimurium, *S.* Enteritidis, *Citrobacter freundii*, *Listeria monocytogenes*, *L. innocua*, *E. coli*, *E. coli* O157:H7, *E. coli* O111:NM, *E. coli* O26:H11, *Serratia marcescens*, *Vibro cholerae*, *Klebsiella pneumoniae*, *K. oxytoca*, *Aerococcus viridans*, *Hafnia alvei*, *and Proteus hauseri*. ELISA results revealed that MAb 6B5 is specific for *Y. pestis*, with the exception of a minor cross-reaction with *Y. pseudotuberculosis*. Western immuno-blot analysis revealed that MAb 6B5 recognizes a *Y. pestis* OMP of ca. 30 Kb.

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