

MICROBIAL STRESS

A SOLID AGAR OVERLAY METHOD FOR RECOVERY OF HEAT-INJURED *LISTERIA MONOCYTOGENES* (Z. Yan, J. Gurtler, and J. L. Kornacki)

A solid agar overlay method was developed for recovery of heat-injured *Listeria monocytogenes*. A pre-solidified non-selective medium, tryptic soy agar with 0.6% yeast extract (TSAYE, 2% agar), was aseptically overlaid onto the top of a solidified selective medium; modified oxford agar (MOX). In principle, injured bacterial cells are resuscitated on the TSAYE overlay before diffusion of selective agents from MOX can inhibit their recovery. A five-strain cocktail of *L. monocytogenes* was heat injured by subjecting the cells to 58°C for 6 min in a water-jacketed flask filled with TSB broth. Both freshly grown and heat-treated cells of *L. monocytogenes* were plated onto TSAYE, MOX and TSAYE/MOX overlaid plates. No significant differences ($P < 0.05$) were found among the three media for recovery of freshly grown (e.g., uninjured) bacterial cells. Selective medium MOX recovered significantly ($P < 0.05$) less *L. monocytogenes* cells than on non-selective medium TSAYE and the TSAYE/MOX overlaid plates. In contrast, there were no significant differences among the TSAYE and TSAYE/MOX overlaid agar plates prepared 0, 2, 4, 6, 8, 16, and 24 h prior to plating heat-injured bacterial cells. TSAYE/MOX overlaid agar was able to differentiate *L. monocytogenes* from a mixture of three additional foodborne pathogens; *Salmonella* spp, *E. coli* O157:H7 and *Yersinia enterocolitica*. This solid agar overlay method for recovery of heat-injured *L. monocytogenes* cells is less time-consuming and less complicated than the conventional overlay/underlay technique and reported thin agar layer overlay methods.

