IAFP Latino 2024
Simpósio Latinoamericano em Segurança dos Alimentos
Santos - SP - Brasil
11 a 14 Nov, 2024

Assessing the performance of three Salmonella ISO 6579-1:2017 validated media using Enterobacteriaceae strains isolated from leafy vegetables grown in Sao Paulo, Brazil

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Salmonella analysis represents an important part of a food microbiology laboratory routine. The isolation step on selective media is the core of *Salmonella* culture-based detection methods, such as the ISO 6579-1 standard methodology. The selection of a suitable isolation agar showing good rates of selectivity, specificity, and productivity is crucial to save time, and to reduce labor and unnecessary costs with multiple confirmatory tests. Therefore, media performance assessment is an important step for laboratories and the use of locally isolated strains, in addition to standards, can reveal more accurate results. This study aimed to assess and compare the performance of three selective media for *Salmonella* isolation regarding productivity, specificity, and selectivity, in compliance with the ISO 11133:2014 guidelines. A total of 87 strains of *Enterobacteriaceae* isolated from leafy vegetables grown in the state of Sao Paulo, Brazil, were used as non-target organisms. These strains were previously identified using the MALDI-TOF MS Biotyper 3.1 database. In addition, *Salmonella* Typhimurium ATCC 14028 and two *Salmonella* spp. strains isolated from leafy vegetables were used as target organisms to assess the performance of Xylose Lysine Deoxycholate (XLD), Hektoen Enteric (HE) and RAPID’*Salmonella* Chromogenic (RS) agars. Bacterial cultures were prepared by growing pure colonies in Brain Heart Infusion (BHI) broth for 24 hr at 37 °C. To assess productivity (recovery level of a target microorganism) and specificity (probability of a non-target strain being correctly classified as negative), each test microorganism was streaked individually in the three plates, aiming to obtain isolated colonies. For selectivity tests (ability to inhibit non-target microorganism), four pools containing four strains each (three non-target strains and *S*. Typhimurium) were prepared by transferring 10 µl of each bacterial culture to a BHI tube. After incubation (37 °C for 24 hr), aliquots of 1 µl were streaked onto plates containing the three different media agars. For all tests, plates were incubated at 37 °C for 24 hr and colonies were investigated according to manufacturers' instructions by two researchers. The three media showed satisfactory productivity by enabling the growth of *Salmonella* strains. Considering specificity, the XLD and HE media showed suspect *Salmonella* colonies for six and 12 non-target bacteria, respectively. In RS, only one of the 87 non-target strains (*Serratia marcescens*) showed suspected *Salmonella* colonies. Additionally, the RS medium demonstrated greater selectivity, allowing significant recovery and differentiation of the target organisms in all four tested mixes. While conventional media operate based on the biochemical characteristics of *Salmonella*, chromogenic media detect target organisms based on specific enzymatic activity, demonstrating greater specificity. Moreover, their formulation includes

selective agents that enhance the inhibition of non-target bacteria, contributing to more effective pathogen isolation. The chromogenic medium demonstrated enhanced specificity and selectivity compared to the conventional media tested. These results underscore the importance of assessing medium performance and highlight the benefits of using chromogenic media as a secondary agar in ISO 6579-1:2017 or alternative methods to improve food safety.

Agradecimentos: