Validation of Most Probable Number technique for determination of *Salmonella* Typhimurium in compost, according to EPA 1682/06

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Abstract

To validate the Most Probable Number technique (MPN) according to the method EPA1682/06 from the U.S. Environmental Protection Agency, in compost and chicken manure, these matrices were inoculated with *Salmonella* Typhimurium ATCC#14028, defining a double blinded study. In order to include all parameters established for a validation process, 504 samples were used: 252 compost samples and 252 chicken manure samples. The detection limit was 0.8 cells/g for compost and 2 cells/g for chicken manure. In addition, the critical level was 5 UFC/g and the correlation between the results obtained from MPN and Absence/Presence techniques, was a high one, showing an r^2 of 0.972.

Key Words

Organic fertilizer, *Salmonella* sp. validation and critical level

Introduction

The development of diseases related to the presence of pathogens such as *Salmonella* spp. in biomaterials and organic manure has brought to consideration the relevance of control measures and above all, it has favored their regulation. This regulation favors the elaboration and application of these agricultural products. The importance of finding detection techniques that allow identification and quantification of *Salmonella* spp. and more so, one which allows avoids effects from interferent microorganisms, permits a guarantee over the results given. The objective of this work was to validate the Most Probable Number technique (MPN) according to the method EPA1682/06 from the U.S. Environmental Protection Agency, in compost and chicken manure inoculated with *Salmonella* Typhimurium ATCC#14028 and to determine the inclusiveness for *Salmonella* spp. after inoculating the samples with 30 different microorganisms (EPA, 2006).

Methods

Using a double blinded study, in order to include all parameters established for a validation process, 504 samples were used, distributed as follows: 252 compost samples and 252 chicken manure samples. These were used for the determination of sensitivity, specificity, relative exactitude, and positive and negative predictive values. Inclusiveness was determined using the critical level and methods correlation with 114 samples distributed in: 88 compost samples and 26 chicken manure samples; these were inoculated with different concentrations of *Salmonella* Typhimurium. For the critical level and the methods correlation finding, samples were inoculated with 5, 10, 20 and 100 cfu/g (colony forming unit per gram), meanwhile, to find the inclusiveness, 100 UFC/g of *Salmonella* Typhimurium were inoculated so they could be processed using the Absence/Presence technique (ICONTEC, 1998) and MPN (EPA, 2006). The inclusiveness was determined by employing 30 interfering strains commonly found in soil.

Results

The reported results for both methods, indicate that, for compost, sensitivity, specificity, relative exactitude, and positive and negative predictive values, a 100% was reported for every parameter, while for the chicken manure a sensitivity of 94.5% , specificity and positive predictive value of 100%, negative predictive value of 96.9% and relative exactitude of 93.7% were obtained, making these results "almost perfect".

The detection limit was 0.8 cells/g for compost and 2 cells/g for chicken manure. In addition, the critical level was 5 UFC/g and the correlation between the results obtained from MPN and Absence/Presence techniques, was a high one showing an r^2 of 0.972.

Considering the inclusiveness, a high recovery of *Salmonella* spp. was found in regard to the 30 strains

employed in this study, since 83.33 % of these overcame the demanded percentage (+/- 30 %), by the NTC 5014:2001 and the remaining 16.67% (commonly reported in the literature as interfering), allowed the recovery of *Salmonella* spp. even if they did not comply with the demanded percentage.

Validation of the method was achieved fulfilling every demanded parameter and it shows a high inclusiveness, obtaining trustworthy results in samples which had a great variety of microorganisms.

References

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