Título: ALTERNATIVE METHODOLOGY FOR AZOSPIRILLUM ENUMERATION IN COMMERCIAL INOCULANTS

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Azospirillum brasilense is a microorganism that presents some difficulties in the development of formulations, especially liquid formulations. It is a microorganism which tends to aggregate and this makes counts highly variable and probably underestimated. There is an official methodology of MAPA for the genera *Azospirillum* which is based on serial decimal dilutions using, as a diluent, saline solution (0.85% NaCl).
We review previous published protocols for *Azospirillum* and other microorganisms with the objective of presenting in this study a variant of the official methodology to improve the reproducibility of *Azospirillum* counts.
Fifteen different packaging of a commercial inoculant containing the strains AbV5 and AbV6 of *Azospirillum brasilense* were used as samples. Serial decimal dilutions using saline solution and the same saline solution plus 0.1 % Tween 80 final concentration were performed for each sample. For spread plate procedure, the dilutions were plated in triplicate, with aliquots of 100 uL, and by the technique of drop plate aliquots of 20 uL were plated in 3 drops for each dilution. The culture media used were the NFb and the "Red Congo" (RC), developed by Rodriguez Cáceres in 1982.
When the technique of spreading was used, the results showed a significant increase in bacterial concentration using saline solution with Tween 80. A significant increase in bacterial concentration was observed using RC medium in relation to NFb medium. The drop plate method also revealed a significant increase in the bacterial counts in relation to the spreading technique.
In conclusion, it is recommended that the Tween 80 diluent solution be used in order to disintegrate the lumps formed by the microorganism. The drop plate technique is an alternative method which is more reproducible than the spreading method. The use of the RC medium presents an alternative that facilitates the distinction between *Azospirillum* and other bacteria, because the colonies are typically stained scarlet red.
We propose this alternative protocol in order to obtain more reproducible results which will be able to be applied in low complexity laboratories including academic, official and private laboratories.

Keywords: *Azospirillum brasilense;* enumeration methodology; inoculants