Title: CULTURE AGE OF *AZOSPIRILLUM BRASILENSE* Ab-V5 INFLUENCES THE INITIAL DEVELOPMENT OF MAIZE

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Abstract:

The bacteria *Azospirillum brasilense* is known to produce biopolymers such as extracellular polysaccharides (EPS) and polyhydroxybutyrate (PHB), and the culture age of this bacterium is crucial to the accumulation of these materials under favorable conditions. These biopolymers can positively influence the survival of bacteria and favour the establishment of root colonization. The aim of this study was to evaluate the initial development of maize roots inoculated with *A. brasilense* Ab-V5 under increasing culture age in a defined culture medium developed to promote the EPS production and accumulation of PHB. The cultivation of bacterial was performed in 3L on a bioreactor using a formula which has its patent pending at INPI. The culture broth was sampled after 12, 24, 48, 72 and 96h of growth, determining the cells density by direct counts and CFU determination. The different culture ages of *A. brasilense* were inoculated, on surface sterilized maize seeds, at a concentration of $1x10^6$ cells per seed, followed a germination assay on "germitest" paper kept in a growth chamber with constant temperature of 28 °C for 10 days. As a control, non-inoculated seeds were used. The following root developmental parameters were evaluated (mean of 10 seedlings): maximum depth (CP), number of roots (NR), roots perimeter (PM), specific length (CE), surface area (AS), roots growth (CM), roots volume (VM) and dry matter (MS). The data were submitted to analysis of variance with application of F test, followed by the Scott-Knott test ($p \leq 0.05$). UFC counts showed maximum after 24h. Significant differences were observed for all the biometric parameters evaluated, showing the influence of the culture age of *A. brasilense* over the growth promotion effect. The 72 hours inoculum provided significant increases in NR, PM, CE, AS, CM and VM which reached up to 80%, 82%, 120%, 47%, 72% and 57% respectively, compared to plants inoculated with 48 hours inoculum. Previous experiments made by our group showed differences in the EPS and PHB content during biomass production for *A. brasilense* Ab-V5, were these biopolymers increases in concentration concomitantly with the culture age. The results presented here indicates that bacterial culture age influences the plant response to beneficial bacteria inoculation, with a potential participation of EPS and PHB content, suggesting that the presence of these biomolecules can enhance the quality of commercial inoculants formulated with *Azospirillum*.

Key-words: Inoculants, plant growth-promoting bacteria, seed germination; bacterial biomass production.

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