

**TITLE:** DEVELOPMENT OF REAL-TIME PCR TO QUANTIFY *Azospirillum Baldaniorum* SP245 ON *Brachiaria* GRASSES UNDER FIELD CONDITIONS

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**ABSTRACT:**

*Azospirillum* genus is a diazotrophic bacteria group that can be inoculated in *Brachiaria* genus and increase plant root and shoot biomass. Efficient methods to quantify the inoculated bacteria are essential for monitoring inoculant efficiency. qPCR assay has been used for the bacterial quantification and is considered as the gold standard for microbial molecular diagnosis. However, the method success depends on the validation of specific primers design for the target bacterium. This study aimed to design and validate a strain-specific primer for subsequent quantification by Real-time PCR of the *A. baldaniorum* Sp245 population in the roots and shoots of *Brachiaria* grass growing under field conditions. Sp245 sequence genome was used to select strains specific regions, to design primers for the target bacteria. Designed primers were evaluated to check the specificity, sensitivity and efficiency. The best pair of primers was used to quantify these bacteria after inoculation in three different cultivars of *Brachiaria* (*Brachiaria brizantha* cv. Paiaguás and cv. Piatã, and *Brachiaria decumbens* cv. Basilik), grown under field conditions. *A. baldaniorum* Sp245 was quantified from extracted DNA of shoot and root tissues by using two different standard curves. The results of qPCR quantification of S245 strain using the selected pair of primers were compared to the microdrop count technique. The results showed that the primer pair Sp245p10 was highly specific to the *A. baldaniorum* Sp245 strain and the quantification by qPCR presented similar results to micro drop count. The qPCR assay using these primers showed that the number of bacteria from *A. baldaniorum* Sp245 was higher in the inoculated plants of *Brachiaria* cultivars, Basilik, and Piatã. Additionally, the number of bacteria observed in the roots was higher than that in the shoots. The qPCR method using a Sp245 strain-specific primer could be used to monitor *A. baldaniorum* used as an inoculant for other plants such as a potential application in field experiments.

**Keywords:** Strain-specific primers, qPCR, Plant growth promoting bacteria, Signal grass

**Development Agency:** CAPES