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MOLECULAR DETECTION OF BACILLUS ALTITUDINIS 19RS3 AND T5S-T4 ISOLATED FROM YERBA MATE (ILEX PARAGUARIENSIS ST. HIL.) USING STRAIN-SPECIFIC PRIMERS

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RESUMO

Introduction: The genus *Bacillus* presents a great diversity of species that are widely distributed in the environment. It is one of the most studied genera and was shown to improve plant growth through a combination of mechanisms. It is used as a biofertilizer that holds promise to make sustainable agricultural practices and ecologically safe. In our previous studies, two endophytic endospore-forming bacteria coded as *Bacillus altitudinis* 19RS3 and T5S-T4 were isolated from *Ilex paraguariensis* St. Hil roots and selected for their plant growth-promoting (PGP) properties *in vitro* and in *vivo*. Strain-specific primers to detect *B. altitudinis* 19RS3 and T5S-T4 strains were designed by whole-genome analysis. Objective: This study aimed to test the pre-designed strain-specific primers with DNA isolated from *B. altitudinis* 19RS3 and T5S-T4. Methods: Genomic DNA from liquid cultures incubated at 30°C for 24 h was extracted by using Sambrook work protocol modified. For the molecular detection, the primers designed for 19RS3: 873F 3´-ATTggCAAAgATAgCAggg-5´, 873R 3´-AgCATCAATCggCTgTggA-5´, 884F 3´-ggTCAgCCTgTAAAAACACCg-5´ and 884R 3´-gTCCCATCCATTAACCTTCA-5´; and for T5S-T4: 2341F 3´-ACACCACATCATTCACTggAgA-5´, 2341R 3´-gCCTTCTAACATCCTgCA-5´, 3296F 3´-gCTACATATCCAACTCCTCAgA-5´ and 3296R 3´-AgCAATAgTAACCgACTTCTCAg-5´ were used. Strain-specific primers were tested in 20 µL standard PCR reactions. The reaction mixture contained 1X Taq DNA polymerase buffer (10X: 500 mM KCl, 100 mM Tris-HCl, pH 9.0 at 25°C, 1% Triton®X-100), 200 µM of each dNTP, 10 pmol of each primer, and 0.5 U of the enzyme Taq DNA polymerase (Inbio Highway, Argentina). Amplifications were performed in a thermal cycler multigene TM II (Labnet International Inc., USA). During the first cycle, DNA was denatured at 94 °C for 5 min. For the subsequent 30 cycles, the tubes were kept at 94 °C for 40 s, at 57 °C for 70 s and at 72 °C for 65 s, followed by an extension at 72 °C for 10 min. The annealing temperatures of 55; 57, and 60 °C were tested. The PCR products were visualized in 2% (w/v) agarose gel stained with GelRed® (Sigma-Aldrich, Germany). The electrophoretic run was performed in an electrophoretic vessel (Electrophoresis Subsystem 70 Labnet International) at 110 V for 30 min and bands were visualized using a UV transilluminator (Model MUV 21-312-220). Results: The primers were specific for each *B. altitudinis* strain, as no amplification products were obtained for negative controls. Amplification products were produced using an annealing temperature of 57 °C. Amplicons of approximately 500

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and 300 bp were generated for *B. altitudinis* 19RS3 and T5S-T4, respectively. Conclusions: *B. altitudinis* 19RS3 and *B. altitudinis* T5S-T4 were successfully detected. The use of strain-specific primers are one of the cheapest and quickest methods to monitoring the colonization of bacterial strains in nursery and field experiments. The strain-specific primers will be applied in future traceability experiments.

PALAVRAS-CHAVE: Biofertilizer, Degenerated primers, Monitoring, Plant growth promoting bacteria, Traceability

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