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SELECTION OF FACTORS AFFECTING THE GROWTH AND INDOLEACETIC ACID PRODUCTION OF BACILLUS ALTITUDINIS 19RS3

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RESUMO

Introduction: Biofertilizers are products composed of living microorganisms that exhibit properties associated with plant growth (PGP), of which the most frequently reported are nitrogen fixation, phosphate solubilization, siderophore production and auxin synthesis. Microorganisms can produce and release auxins as secondary metabolites, with indoleacetic acid (IAA) being the most frequently reported. The IAA is synthesized from L-tryptophan, but tryptophan-independent synthetic pathways are also described. One feature limiting the application of biofertilizers is obtaining an enough amount (growth) of the endophytic bacteria or the product of interest in a short period of time. Objective: This study aimed to assess the influence of several factors on the growth and production of IAA of *Bacillus altitudinis* 19RS3, a PGP bacteria isolated from yerba mate (*Ilex paraguariensis* St Hil.) roots. Methods: A half-fraction experimental design with six factors and two levels was used. The variables growth medium (nutrient broth or trypticase soy broth), initial pH (7.0 or 7.5), temperature (31 or 34 °C), agitation (static or 120 rpm), incubation time (24 or 48 h), and addition of L-tryptophan (0 or 100 ppm) were evaluated. *Azospirillum brasiliense* sp. 245 was used as positive control. Flasks containing 15 mL of the corresponding medium, pH value, and IAA concentration were prepared. Twenty-four hours old stock cultures were adjusted to 0.5 of McFarland's scale and 150 µL were added to each flask. Flasks were incubated at the corresponding temperature, time, and agitation speed according to each experimental run. The growth was determined by measuring the optical density (OD) at 600 nm in a spectrophotometer. Production of indoles was determined by a colorimetric method at 535 nm using Salkowski's reagent. The concentration of indolic compounds in µg mL^{-1} was calculated using a concentration curve for indoleacetic acid. The experimental matrix for the screening (consisting of a set of 32 runs) was designed and analysed using the software Statgraphics Centurion XV. Factors with p-values of less than 0.05 were considered to have significant effects on the response (growth or indole synthesis). Results: For *B. altitudinis* 19RS3, agitation increased growth, while IAA production was increased by the addition of L-tryptophan and incubation time, and decreased by temperature. The highest growth (OD of 2.49) was registered using trypticase soy broth, initial pH of 7.5, 34 °C, agitation, without addition of L-tryptophan at 24 h, while the lowest growth (OD of

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0.39) was registered using nutrient broth, pH of 7.5, 34 °C, agitation, without L-tryptophan at 48 h. The highest IAA production (32.39 µg mL^{-1}) was obtained using nutrient broth, pH of 7.5, 31 °C, agitation, addition of 100 ppm of L-tryptophan for 48 h. The lowest IAA value (0.22 µg mL^{-1}) was obtained using nutrient broth, pH of 7.0, 34 °C, agitation, addition of 100 ppm of L-tryptophan for 48 h. Conclusion: Identification of these factors will allow us to proceed with the optimisation of the growth conditions for *B. altitudinis* 19RS3, which is key for reducing production costs in the development of a bioproduct for stimulating the growth of yerba mate plants.

PALAVRAS-CHAVE: Bacillus, factorial design, optimisation, PGPR, screening

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