DEVELOPMENT OF A HIGH-THROUGHPUT SCREENING METHOD FOR PLANT-GROWTH PROMOTING BACTERIA

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Traditional bacterial screening methodologies tend to be expensive and time-consuming. Taking it into consideration, we adapted reference protocols and developed a miniaturized methodology to optimize the cultivation and selection of strains with potential characteristics of plant-growth promotion. All bacteria were cultured in polypropylene 96-deepwell plates, which can be autoclaved and reused. To fill the plates, a low cost device, such as the Vaccu-Pette/96™ (Sigma-Aldrich) can be used. The plates must be closed with breathable membranes that allow gas exchange, such as *Breathe Easier Sealing* (Sigma-Aldrich). For greater control, it is recommended to have technical replicates for every strain and uninoculated wells as negative growth controls. Inoculation between plates can be quickly performed using multichannel pipettes. Biochemical or physiological tests such as indoleacetic acid (IAA) production, phosphate solubilization, halotolerance or the ability to grow using a certain substance can be easily done in the same system by changing the growth medium. The developed method was validated with the cultivation and selection of 321 strains isolated from the leaves of the plant *Atriplex nummularia*. The characteristics we screened for were the presence of *nifH* and *acdS* genes, and the production of AIA. For molecular analysis, DNA extractions were performed directly from the plates, using the Wizard® Genomic DNA Purification Kit (Promega), adapting the protocol to an initial volume of 200µl and the centrifuge speeds to those of a 96-deepwell plate. The detection of the *acd*S gene was performed by qPCR and the *nifH* gene by PCR followed by gel electrophoresis. For AIA quantification, 96-well deepwell plates with King's B medium were inoculated. After 72h of incubation the plates were centrifuged and 80µl of the supernatant was combined with Salkowski's reagent, centrifuged again, and transferred to a 96-well microtitration plate. The reading was taken in a spectrophotometer at 530nm, together with a standard curve, which allowed a simultaneous quantitative analysis of 40 bacteria, in duplicate. At the end of the screening, 12 *nifH*+, 52 AIA+ and 7 *acdS*+ strains were obtained. The adoption of this method represented a great gain in scale, savings, and time, and it is an accessible method for most laboratories.

Palavras-chave: HIGH-THROUGHPUT SCREENING, PGPB, biotechnology.

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