

DETERMINATION OF THE TITER OF A BACTERIAL SUSPENSION OF *Bacillus subtilis* CNMN-BB-06

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Introduction. The use of antagonistic microorganisms is an environmentally friendly approach to biological plant protection. Among them, *Bacillus subtilis* is of particular interest due to its ability to produce antifungal metabolites and suppress phytopathogenic microorganisms. For its effective use in biological research and microbial bioproduct development, determination of the bacterial suspension titer is essential to standardize the concentration of viable cells.

Aim of the study. To obtain a bacterial suspension of *Bacillus subtilis* CNMN-BB-06 and determine its titer using the plate-count method on a nutrient medium followed by colony enumeration.

Materials and methods. The study was carried out in the Laboratory of Phytopathology and Biotechnology, Institute of Genetics, Physiology and Plant Protection. The strain *Bacillus subtilis* CNMN-BB-06 was cultivated by submerged fermentation on a microbiological shaker at 200 rpm for 70 h in a liquid nutrient medium. The titer of the obtained bacterial suspension was determined by preparing a series of tenfold serial dilutions up to 10^{-6} . An aliquot of 0.25 mL from the selected dilution was transferred with a sterile pipette onto a Petri dish and evenly spread over the agar surface using a sterile Drygalski spatula in the first plate, followed by the second and third plates using the same spatula. The plates were incubated at 30°C for 48 h, after which the colonies grown on the three plates were counted. The bacterial titer was calculated using the following formula: $M = (a \times 10^n) / V$, where M is the bacterial titer (CFU/mL), a is the number of colonies, 10^n is the dilution factor, and V is the inoculum volume.

Results and discussion. Submerged cultivation of *Bacillus subtilis* CNMN-BB-06 in liquid nutrient medium resulted in a homogeneous bacterial suspension suitable for plant protection applications by fine-droplet spraying. Titer determination of the selected dilution using an inoculum volume of 0.25 mL yielded 70 colo-

nies. According to the calculation, this corresponded to a bacterial titer of 0.3×10^9 CFU/mL. The obtained value indicates a high density of viable bacterial cells and confirms the effectiveness of the cultivation conditions. Standardization of the bacterial suspension is an essential step in ensuring experimental reproducibility and providing a reliable inoculum for further studies on the biological activity of the strain against phytopathogenic microorganisms.

Conclusions. Submerged cultivation enabled the production of a *Bacillus subtilis* CNMN-BB-06 bacterial suspension with a titer of 0.3×10^9 CFU/mL. The obtained results provide a standardized suspension with a high concentration of viable cells and can be used to establish optimal application rates for the biological protection of plants against harmful organisms.

Keywords: *Bacillus subtilis* CNMN-BB-06, bacterial titer, serial dilution, submerged cultivation.

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