PERFORMANCE EVALUATION OF THE FERMENTATION PROCESS OF BACILLUS *SUBTILLIS* AND *MEGATERIUM*

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ABSTRACT

In modern agriculture there is a need to produce more and more food, minimizing operational costs and reducing impacts on the environment as much as possible. The use of biological control is a strategy that man has been using for years to manage natural enemies, seeking to control pathogens and pests. Seeking more sustainable alternatives, the use of bacteria gains prominence and the use of Bacillus species as growth promoters and biological control agents for various agricultural crops provides an attractive, efficient and environmentally less aggressive method than pesticides and chemical fertilizers, which makes the practice of using bioagents a more sustainable alternative in the economic and environmental spheres. However, the costs in relation to the culture medium can be an obstacle. Seeking to solve this problem, the objective of this study was to test a commercial inoculum medium for the growth of Bacillus *subtilis*. To achieve this objective, tests in a shaker incubator, a benchtop bioreactor linked to the serial dilution technique, were used in the study. In an incubated shaker, the tested medium proved to be very promising for the multiplication of Bacillus *Subtillis.*

.Keywords:1.*Megaterium* 2.*Subtillis* 3.controle biologico

1 INTRODUCTION

The market for bioinputs for plant nutrition, as well as biocontrol, is growing worldwide and is projected to continue growing at rates above 10% per year in the coming years. In this context, bioinputs for biological control are considered important technologies for reducing the widespread use of agrochemicals, with important results to mitigate the social, environmental and economic issues mentioned (MORAES, 2019).

The use of plant growth-promoting microorganisms to increase agricultural production is an important tool today in the world. This is due to the great demand to reduce dependence on chemical fertilizers and the need to develop sustainable agriculture (MACHADO et al., 2012). Low-cost inoculants with plant growth-promoting microorganisms are an alternative to reducing environmental risks caused by inadequate, and sometimes excessive, use of inputs and pesticides. Plant growth promoters also contribute to increasing agricultural production, making the product more competitive and differentiated, and also reducing costs for the producer (BRAGA JUNIOR et al., 2018).

These microorganisms play a fundamental role in soils, especially in the carbon cycle and the genesis of humic substances. The exudates released by microorganisms and plant roots contribute to the establishment of proton and electron activity in the soil solution and also to the recycling of soil trace elements. Of the exudates, formic, acetic, oxalic, tartaric and citric organic acids appear among the most characterized.

Bacteria of the genus *Bacillus* can be considered plant growth-promoting rhizobacteria, as they have the ability to colonize the rhizosphere and promote plant growth (KUNDAN et al., 2015), are essential for nutrient recycling and have potential as biofertilizers to increase productivity, in addition to being able to benefit the plant during periods of stress (KAVAMURA et al., 2013. *B. subtilis* is a soil bacterium that produces hormones such as indole acetic acid (IAA) and indole butyric acid (IBA) (ARAUJO et al ., 2005) and secretes important enzymes for plant nutrition (POWAR & JAGANNATHAN, 1982). CHAGAS JUNIOR and collaborators (2022) studied strains of Bacillus subtilis as an inoculant that promotes plant growth in soybeans. solubilize phosphate and produce indole acetic acid (AIA). The *B. subtilis* isolates showed efficiency in the biomass variables of the aerial and root parts of soybean plants, with emphasis on the *B. subtilis* Bs10 isolate. Strains BRM 119 *(Bacillus megaterium)* and BRM 2084 *(Bacillus subtilis)* initiate the production of different organic acids. These acids act on the portion of the soil that is in contact with plant roots, thus starting the process of solubilization of phosphorus that is retained in the calcium, aluminum and iron present in the soil, leaving it readily available for absorption and assimilation by the plant.

2 MATERIAL & METHODS

The methodology followed was based on the Manual for production and quality control of biological products based on bacteria of the genus *Bacillus* for use in agriculture, from the Brazilian Agricultural Research Corporation – Embrapa, May 2020. The experiments were carried out in 250-liter Erlenmeyer flasks. mL using 100 mL of culture medium. All treatments were carried out in duplicates and the culture media had their pH corrected to 7.0 and sterilized for 25 minutes at 121ºC in an autoclave. In preliminary tests, aliquots of the pure product were used to inoculate the Erlenmeyer flasks. After inoculation, the initial concentration of cells in the medium was determined in each container. The working conditions of the shaker were a temperature of 30ºC and agitation of 175 rpm.

The culture medium used was the PRO 100 medium from the Company Innovar and also a synthetic culture medium containing, . After defining the culture medium for each microorganism, reagents of economic importance will be selected to optimize their concentrations. To evaluate the cellular growth of microorganisms, the serial dilution technique described by Tortora (p. 166-169, 2017) was used with modifications to the scale, using 0.1 mL of sample for 0.9 mL of solution in the dilution. saline 0.85%. Hicrome techniques were also applied to identify microorganisms.

3 RESULTS & DISCUSSION

All fermented broths were analyzed by microscopy, showing good cell growth for all microorganisms. *Bacillus subtilis* was the one that presented the best performance, reaching an order of magnitude of $1{,}0\times10^{9}$ spores/mL with a good formation of dispersed spores in both fermentations, but the presence of vegetative cells was also noted.

result of Bacillus Megaterium and subtilis fermentation

Pro100:

Microrganismos	UFC médio Inicial (esporos/mL)	UFC médio Final (esporos/mL)	pH Final
B. subtilis	1×10^{2}	9×10^{8}	6,42
B. megaterium	8×10^{2}	3×10^{10}	4,33

Sintético

Microrganismos	UFC médio Inicial (esporos/mL)	UFC médio Final (esporos/mL)	pH Final
B. subtilis	1×10^{7}	$3{,}30\times10^{9}$	6,27
B. megaterium	6×10^{7}	$1{,}20\times10^{9}$	5,12

Image 1: Hi-Crome from the Microorganism *Subtilis* (CNPMS B2084)

Image 2: Hi-Crome from the Microorganism *Bacillus Megaterium (CNPMS B119)*

4 CONCLUSION

The objective of the work was to test a synthetic medium and a commercial medium using *Bacillus subitilis* and *Bacillus Megaterium* to observe the behavior of both, to evaluate their multiplication and analyze a more accessible medium.

It was proven in this work that the best performance of the microorganisms *Bacillus subitilis* and *Bacilus Megaterium* was in the commercial environment (Pro 100) which becomes more viable for the farmer, proving that it had an initial magnitude of $8x10^{2}$ and ending $10x10^{10}$.

Bacillus Subitili has an important role in agriculture, helping with soil and high crop productivity, and has several biotechnological products such as enzymes, antibiotics and probiotics, in addition to its potential in nitrogen fixation, *Bacillus Megaterium* has the ability to decompose matter organic in addition to producing cellulase and amylase enzymes that are involved in the decomposition of plant residues and the solubilization of minerals, which makes it beneficial for stopping plant growth and increasing their resistance to biotic and abiotic stresses, and is also studied as a nematicide.

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