SELECTION OF CULTURE MEDIA FOR IN VITRO PRODUCTION OF RHIZOBACTERIA

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ABSTRACT

The use of living organisms through biological control represents an efficient approach to agricultural pest management, in addition to being ecologically sustainable. Global research has increasingly shown the great importance of microorganisms in agriculture, aiming to increase production, vegetative efficiency and reduce costs. One of the most used biological agents are bacteria of the genus Bacillus, which stand out for their production of enzymes that inhibit pathogenic development. The present work aims to explore the growth of these microorganisms in laboratory space, before being taken to the field. However, appropriate culture media are required for experiments with Bacillus, resulting in an obstacle, as the costs in relation to the media can be high. To solve this problem, tests were carried out with two types of lower-cost commercial inoculum, in order to identify the most efficient one for such an experiment. In the PRO 100 E1 culture medium, growth was more promising for the multiplication of microorganisms.

.Keywords: 1.*Bacillus* 2.*Microorganisms* 3.*Pathogenic*

1 INTRODUCTION

Agricultural production can be influenced by microorganisms in different ways, such as by promoting plant growth, inducing resistance against pests, pathogens, among others. To explore the benefits generated by microorganisms such as rizhobacteria, techniques are needed for *in vitro* production of isolated and selected species, carrying out the cultivation of such microorganisms in culture media that enable their growth and multiplication of cells, so that it becomes feasible to use them for colonization of seeds, roots or even the plant itself and, Verification of your benefits such as beneficial microbial agents in the production process or plants protector.

These bacteria are present in the rhizosphere, revealing large versatility in terms of the nutrients required by them, which can contribute to a competitive advantage over the other groups of microorganisms they colonize specific habitats along the root (BURR and CAESAR, 1985). Growth promotion can be the result of several mechanisms, such as: biological control by competition for nutrients with the pathogen; production of siderophores and antibiotics; disease-induced resistance and growth promotion directly by phytohormone production and increased nutrient availability by nitrogen fixation or phosphorus solubilization (KLOEPPER, 1993; NEHL et al., 1996; WHIPPS, 2000)

Considering that culture media used in laboratories on a smaller scale, in research with such microorganisms, are very expensive and become unfeasible for commercial use, the obtaining of less expensive culture media that make the production of the microorganisms, which are currently used on a large scale to improve production processes of cultivable and commercially exploited plant species such as Corn, Soybean and Cotton. The bacterial genera that sand out the most as growth promoters are Pseudomonas, Bacillus, Serratia, Azospirillium and Azotobacter (ZAADY et al., 1993; Rodríguez and FRAGA, 1999).

To produce microorganisms of different species, after their isolation in different environments, different culture media are tested to verify nutritional adequacy, biosynthetic capacity, among others, in the laboratory (MADIGAN et al., 2004), however, for the production of microorganisms on a large scale commercial, adaptations of culture media are necessary to reduce costs, without loss of nutritional quality, keeping the microorganism with a high potential for colonization.

2 MATERIAL & METHODS

The experiment will be carried out at the Entomology Laboratory (LE-IFTM) of the Federal Institute of the Triângulo Mineiro – Uberaba Campus, in microbiology, in a biological grade laminar flow chamber, using sterile materials, under temperature, relative humidity and photo phase, controlled according to the needs of different microorganisms. All materials used will be sterilized in an autoclave, following the necessary precautions. The treatments will consist of 2 culture media provided by a partner company, however, without knowledge of its formulation that follows through industrial secret: PRO 100E1, PRO 100E2.

The bacteria that will be used in the experiment belong to different genera, supplied by a partner company, following the same industrial secret situation – isolates: GRA 801, GRA 802, GRA 803, GRA 804, GRA 805 and GRA 808. With all the items necessary fot the experiment in the laboratory prepared and made available, fermentations will be carried out in Erlenmeyer with 50mL of each medium, incubated in a Shaker (agitation and aeration), with temperature controlled at 30ºC for a period of 48 hours. Subsequently, serial dilutions will be performed, ranging from 1 to 14 times (10^{-1} a 10^{-13}), using 3 dilutions, according to the previous observation of bacterial growth (multiplication), to obtain and count isolated colony-forming units (CFU), by plating these dilutions in NA (Nutrient Agar) culture medium.

3 RESULTS & DISCUSSION

All fermented broths were diluted and analyzed by microscopy, showing good cell growth in both (PRO 100E1 and PRO 100E2) for the microorganisms GRA801, GRA802 and GRA805. By analyzing it, it is concluded that the two-culture media are good, varying according to the need of each microorganism, but PRO100 E1 proved to be more effective.

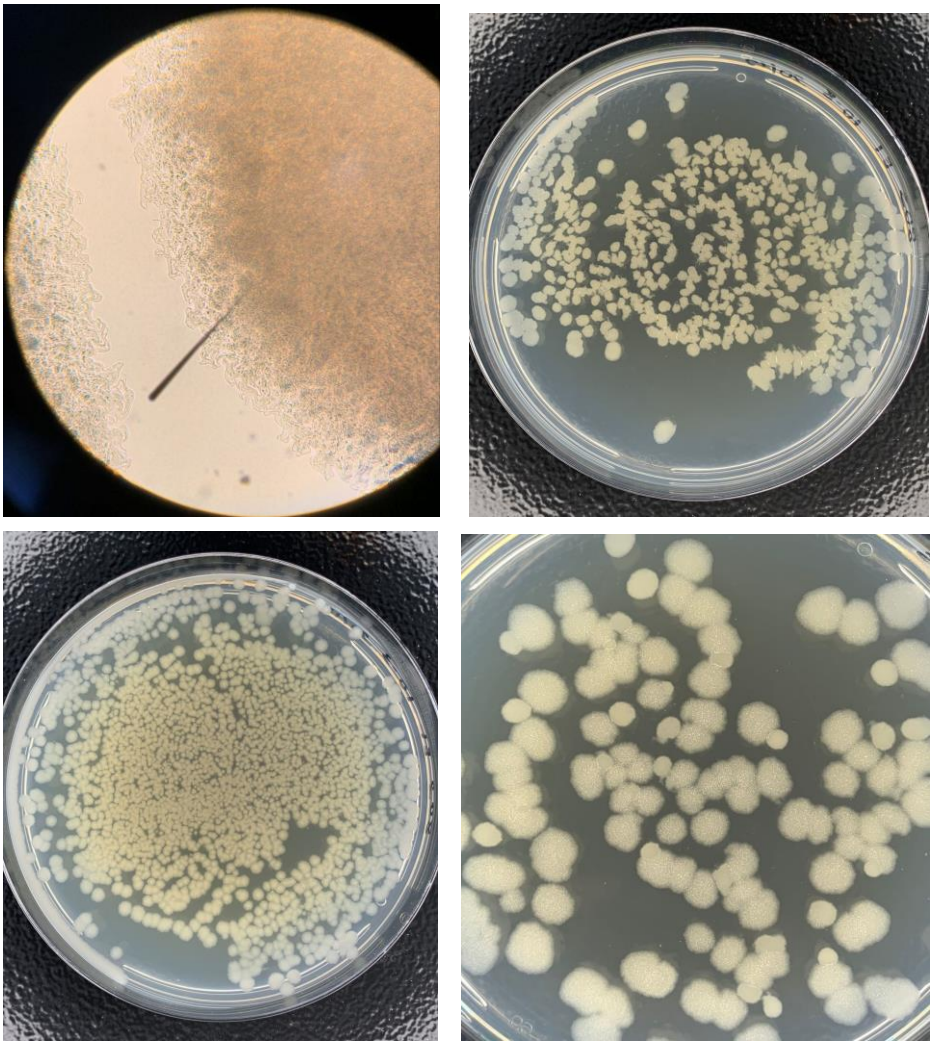
Pro100 E1:

Microorganisms	UFC medium Finally (spores/mL)
801	2,2x10^11
802	3,4x10^9
803	3,1x10^8
804	2,5x10^9
805	2,6x10^9
808	2,9x10^12

Pro100 E2:

Microorganisms	UFC medium Finally (spores/mL)
801	1,3x10^7
802	2,4x10^6
803	1,6x10^5
804	1,9x10^7
805	2,8x10^7
808	3,0x10^15

Images



Growth check for manual counting of GRA808 microorganism at different dilutions and analysis under the microscope.

4 CONCLUSION

Most of the microorganisms used in this work demonstrated performance in both culture media (PRO 100E1 and PRO 100E2), however, in the PRO 100E1 medium, the replication of microorganisms was greater. Both are cheaper means for the process of inoculating microorganisms, which becomes a great benefit for large-scale production, such as in companies. Other types of culture medium are recommended for those microorganisms that did not grow during the research.

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