BIOENCAPSULATION AS AN ALTERNATIVE TO MAINTAIN MICROBIAL INOCULANTS VIABILITY AGAINST ULTRAVIOLET EXPOSURE

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ABSTRACT

The encapsulation is a form to protect plant growth-promoting bacterias against external factors, stabilize and improve their viability. The purpose of the present work was to analyze cell viability of non-encapsulated and alginate encapsulated *Bacillus amyloliquefaciens* LB02 strain when exposed to UV light. The encapsulation was done using 1% sodium alginate and sodium chloride, through an extrusion method. The capsules and an inoculum with free bacteria were exposed to different times of UV light, and the survival rate analyzed using CFU counting. The results indicated that after 8h hours of UV light exposure the free inoculum did not survive, and the encapsulated maintained high cell countings. The results indicate that encapsulation could be an alternative to protect bacterias applied to plants from the sunlight, increasing the shelf life and effectiveness of biological products in field conditions. Additional research could explore different encapsulating materials and application methods, seeking to optimize protection against sunlight and other adverse environmental factors.

Keywords: *Bacillus amyloliquefaciens*. UV protection. Plant-growth Promoting Bacteria. Sodium Alginate.

1 INTRODUCTION

Plant growth-promoting bacteria (PGPB), often derived from the plant's rhizosphere, has a high potential to aid in plant growth and vitality, facilitating nutrient solubilization and acquisition. Additionally, its ability to synthesize hormones, perform nitrogen fixation, and control of plant pathogens praises their multifunctionality and has placed these bacteria at the top of biological products applied to agriculture[1]. The bacterium *Bacillus amyloliquefaciens* is a PGPB that assists the plants with nitrogen fixation, phosphate solubilization, siderophore production and phytohormone production. In addition, it produces antimicrobial compounds such as hydrogen cyanide (HCN) and cyclic lipopeptides like surfactin, which are utilized to impede the growth of pathogenic microorganisms[2]

However, it is known that when applied to plants in the form of inoculants, the bacterias are exposed to external factors such as rain, sunlight, lack of nutrients and competition with other microorganisms, resulting in a rapidly decrease of their survival rate[3,4]. The sun emits UV radiation with three different wavelengths (λ), namely UVA (λ = 320−400 nm), UVB (λ = 280−320 nm), and UVC (λ = 100−280 nm), however, the radiation that reaches the atmosphere is only UVA and UVB, since UVC rays are retained in the ozone layer[5]. This spectrum may have a large impact on the survival and longevity of any microorganism deposited on the stems and leaves of plants[6].

The encapsulation is a form to protect the bacterias of external factors, and is based on producing a continuous coating around a matrix in which bacterias are present[7]. It can be formed using polymers like sodium alginate, found in the cell walls of brown algae, that is a way of gum that reacts with calcium salts or acids and forms irreversible gels. This method aims to stabilize cells, shield them from abiotic and biotic stresses, and potentially improve their viability and stability. In addition, encapsulated bacteria can be released in a controlled and slow way to the soil, therefore providing beneficial effects for a long time on plant development under unfavorable conditions[8].

Considering the beneficial effects of cell encapsulation, this work aims to test the viability of non-encapsulated and alginate encapsulated *Bacillus amyloliquefaciens* LB02 strain when exposed to UV light. This study seeks to determine if encapsulation could serve as an effective method to protect bacteria applied to plants from sunlight exposure.

2 MATERIAL & METHODS

For the production of capsules, initially, the inoculum of the rhizospheric *Bacillus amyloliquefaciens* LB02 strain was prepared in Luria Bertani (LB) liquid medium, and grown for 24 hours at 37°C and 150 rpm. Then, 10 mL of the inoculum was mixed with 10 mL of 2% sodium alginate, and 100 µL of the bacteria-alginate solution was dripped, using a micropipette, into a 0.1M calcium chloride solution under constant agitation. The formed capsules were left for 3 minutes under agitation in the solution, then was removed, washed with sterile distilled water and transferred to filter paper to remove excess water.

To perform the analysis of bacteria survival under UV light exposure, Petri dishes with 100 µL of capsules and Petri dishes with 100 µL of free inoculum were used. They were exposed to radiation in a cabinet with a 20 ampere UV-A lamp at a distance of 20 cm. Every 2 hours, two plates, one with inoculum and one with capsules, were removed to analyze bacteria survival. Serial dilution was performed on each one, and with samples without UV light exposure. For the inoculum, dilution was done by adding

900 µL of saline solution to the plate. For capsule dilution, after exposure, they were placed in Eppendorf tubes and 900 µL of 1.6M sterile potassium phosphate was added to dissolve them and release their internal content. After 48 hours of capsule dissolution, serial dilution was performed and plates incubated for 16 hours at 37°C. The CFU/mL was then determined by counting the colonies.

3 RESULTS & DISCUSSION

Through the experiment of different times of UV exposure to the non-encapsulated and alginate encapsulated *Bacillus amyloliquefaciens* LB02 strain, satisfactorily results were obtained, maintaining cell viability through UV exposure when using encapsulating approach (Figure 1) .

The bacteria population in alginate capsules slowly decreased over time of UV light exposure, however, even after eight hours, cells remained viable inside the capsules, reaching 1 x 10^5 CFU/mL. On the other hand, free bacteria that received direct light completely lost their viability after 6 hours of exposure, when no growth was detected.Thus, encapsulated *Bacillus amyloliquefaciens* with alginate microcapsule has been proven to be beneficial to the increased resistance to UV radiation, however, other formulations can be tested to optimize this resistance.

The use of a more concentrated alginate solution can be a good solution to improve the time of resistance, but it's necessary to analyze if the highest concentration will not disturb the release of the cell and the use of the extrusion technique and cell release in soil. Furthermore, other components like clays, proteins and carbohydrates also can be added to the capsules to have nutritional properties for microorganisms, enhance bacterial survival under unfavorable conditions and reduce the adverse effects of drying[9].

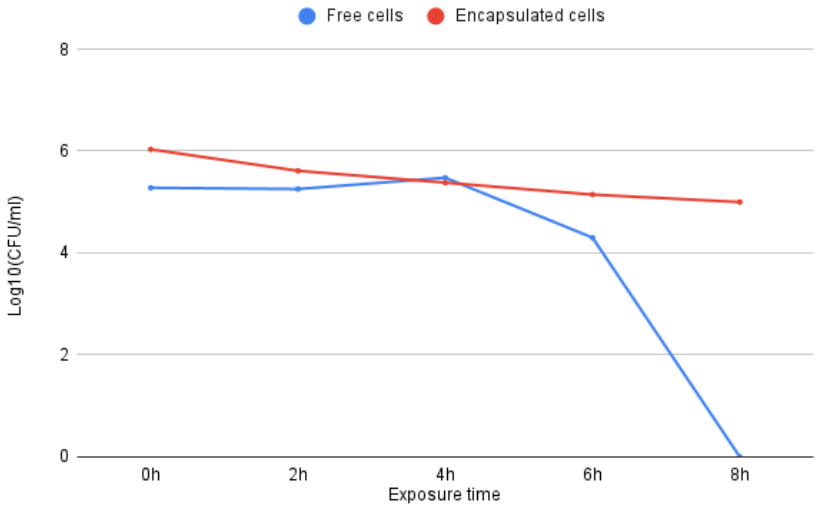

Figure 1 Viability of non-encapsulated and alginate encapsulated *Bacillus amyloliquefaciens* LB02 at different exposure times of UV radiation.

4 CONCLUSION

The encapsulation of *Bacillus amyloliquefaciens* LB02 with sodium alginate could improve cell survival, adaptation and protection against field stress. Evaluating the viability of non-encapsulated and alginate encapsulated cells against different times of UV exposure (0h, 2h, 4h, 6h, 8h), we concluded that the capsules are efficient to protect the bacteria for at least 8 hours of exposure.

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