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Short Communication

Influence of the production season on the microbiological stability of Azofert®-S biofertilizer

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ABSTRACT

The objective was to evaluate the concentration of strain *Bradyrhizobium elkanii* ICA 8001 strain in Azofert®-S inoculants produced at different year seasons. Inoculants were produced in the four seasons of the year and stored at room temperature. Purity and bacterial strain concentration were determined every 30 days for 180 days. Inoculants produced in fall and winter maintained a strains concentration 10^8 CFU mL^{-1} for 120 days, which is optimal for field use. Knowing the effectiveness time of Azofert®-S would establish an inoculant production strategy that ensures the biofertilization of soybean.

Key words: *Bradyrhizobium*, concentration, quality, temperature

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a legume that establishes a mutualistic association with concentration of rhizobia, which allows it to assimilate height nitrogen concentrations that it needs to satisfy nutritional requirements [(1,2)]. This crop has a notable economic value since it constitutes a highly proteinaceous food for animal and human diet [(3,4)].

Given the contribution made by rhizobia in the nitrogen nutrition of soybean, this crop is biofertilized in a great part of the world [(5)], so that several biofertilizers are commercialized for this purpose [(5,6)]. An example is Azofert®-S, a biofertilizer with

bacterial strain *Bradyrhizobium elkanii* ICA 8001 and nodulation factors, which is used as part of the integrated management of soybean [7].

In biofertilizer production, quality control is fundamental for the optimal product performance. The microbial culture purity and cell concentration above 1 x 10^8 CFU mL^{-1} (Colony Forming Units per milliliter of inoculant) are main indicators that determine its quality [8]. It is necessary to establish the effectiveness time of the inoculant under storage conditions, which is very important thing to maintain the quality of the product and its permanence in the market. The objective of the present study was to evaluate the viability over time of strain *B. elkanii* ICA 8001 in the biofertilizer Azofert®-S, when it is produced at different year seasons and kept at room temperature.

MATERIALS AND METHODS

The strain *B. elkanii* ICA 8001 was used, coming from the strains collection of the Microbiology Laboratory of the Plant Physiology and Biochemistry Department, INCA. Inoculants of this strain were prepared at four times: summer (August-February), (October-April), winter (December-June) and spring (May-October), between the years 2014-2016.

Pure colonies of the strain, preserved at 4 ºC in Yeast-mannitol (YM) medium [9], were inoculated into Erlenmeyers containing 10 mL of sterile Bradyfact culture medium [7]. The flasks were kept under shaking conditions at 130 rpm and 28 °C, for 72 hours. The bacteria growth was scaled at 1:10 (v/v), under the same conditions of agitation and temperature, and 1000 mL of inoculant was obtained. In all cases, flasks whose inoculum volume represented one fifth of total volume of the flasks were used to guarantee an aerobic environment. The inoculants were packed in five sterile flasks (240 mL) and stored at room temperature.

When inoculants were prepared and every 30 days, during 180 days of conservation, three flasks were chosen at random and the purity of cultures was determined by Gram staining. For this purpose, morphological characteristics of bacterial cells, response to staining, presence of endospores and presence of contaminating microorganisms were taken into account [10]. In addition, cell concentration was determined, for which 1 mL of inoculant was taken and serial decimal dilutions were made. One hundred microliter were cultivated on plates with solid YM medium and incubated at 30 °C, seven days. The number of CFU mL^{-1} was then determined, according to the expression:

$$CFU\ mL^{-1} = No.\ col \times 10^{-1} \times d$$

where:

No. col: colony number

d: dilution factor

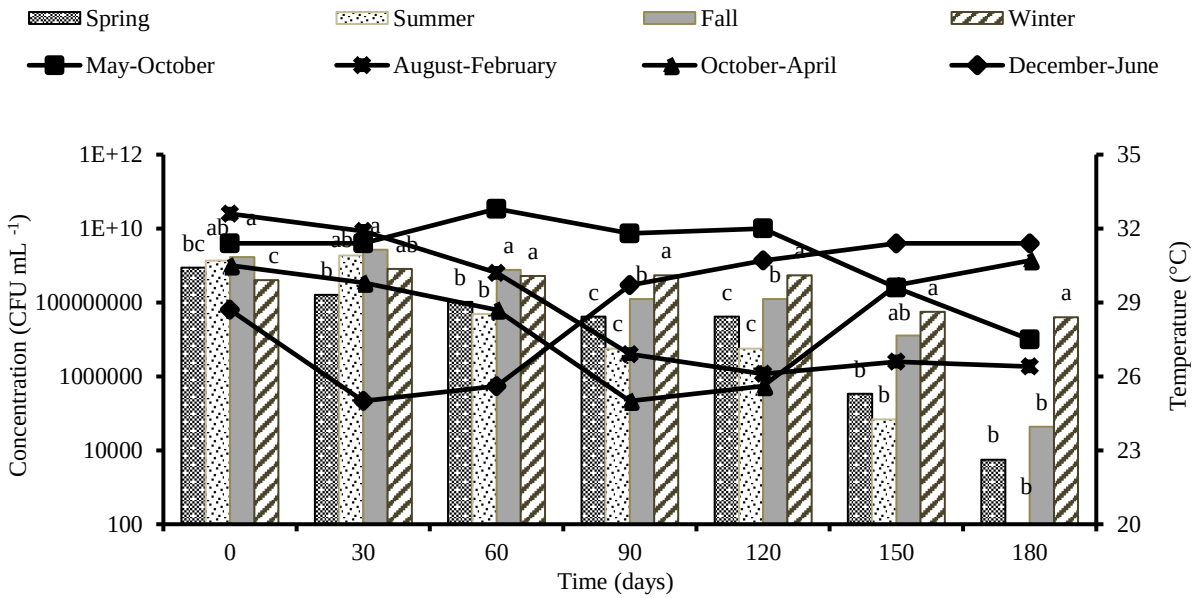
STATISTICAL ANALYSIS

Cell concentration data were processed by simple rank analysis of variance. Tukey's mean comparison test for $p<0.05$ was used to discriminate differences between treatments, which corresponded to different inoculant production periods [11]. Data were processed in Statgraphics Plus version 5.1, 2001 and plotted in Microsoft Excel, 2016.

RESULTS AND DISCUSSION

Purity evaluations showed, only Gram-negative bacilli, without endospores, which are typical characteristics of strain ICA 8001 [7].

The strain viability in Azofert®-S inoculant during storage in different periods is shown in Figure 1. The strain maintained concentrations of 1×10^8 CFU mL^{-1} during the first 30 days of storage, in all season of the year that it was produced. This concentrations are adequate for use the inoculants [12]. The storage temperature during this period was between 25-32 °C, according to different seasons.



Statistical analysis was performed at each evaluation time

In the bars, means with same letters do not differ statistically (Tukeyp≤0.05, n=3)

Lines represent temperature behavior in the different seasons

Figure 1. Viability of strain *B. elkanii* ICA 8001 in Azofert®-S biofertilizer, produced and stored for 180 days at room temperature

The inoculants produced in fall and winter had a cell concentration of 10^8 CFU mL^{-1} up to 120 days, while those produced in spring only had this concentration up to 60 days. Those produced in summer did not have that bacterial concentration beyond 30 days.

At 120 days, the inoculants produced in winter had a higher bacterial concentration than those prepared in fall, the inoculants prepared in winter and fall were subjected to lowest temperatures during the first 60 days. It is (25.6-28.7°C and 28.7- 30.5°C respectably. The bacterial inoculants conservation in temperature below 30 °C, showed that it is possible to maintain the bacterial concentration without significant variations [13].

However, inoculants produced during the spring, in the first two months of conservation were subjected to 31.4 °C, which increased in the next three months. This could explain the decrease of bacterial viability. The inocula that were produced in summer were maintained to temperatures above 31 °C, the highest at the time of produce of all inoculants. The negative effect of temperature on the preservation of inoculants is known [13].

Despite the negative influence of temperature on strain preservation, especially at the moment of product processing, it could be appreciated that time storage produce an unfavorable effect on the bacterial viability. Similarly, other authors report that storage time is an important factor to be taken into account in the survival of species such as *Sinorhizobium meliloti* [14], *Rhodotorula mucilaginosa* [15] and *Pseudomonas fluorescens* [16].

CONCLUSION

The stability over time of the strain *B. elkanii* ICA 8001, present in Azofert®-S inoculants, depends on the year season in which they are produced, which is due to their sensitivity to exposure to temperatures above 31 °C.

RECOMMENDATIONS

- To produce soybean inoculants in fall and winter seasons and if it is necessary to store them at room temperature, it should not be for more than 120 days. If they are produced in spring or summer, they should be used in the first 60 and 30 days, respectively.
- To evaluate the effect of preservative compounds in the inoculant to extend the effectiveness time of the product.

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