

EFFECT OF CELL PROTECTANTS ON MASS MULTIPLICATION OF *AZOTOBACTER*

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ABSTRACT

The liquid bioinoculant formulation technology goals to solve the drawbacks associated with solid carrier based formulation like shorter shelf life, high contamination, poor quality, low field performance and processing the carriers. This study was conducted to formulate and determine the effect of liquid biofertilizers containing different cell protectants on the strain *Azotobacter chroococcum*. The cell protectants used were glycerol (1%), polyethylene glycol (PEG, 2%), Polyvinyl alcohol (PVA, 0.5%) and Gum Arabic (GA, 0.3%). The 2 treatments without addition of cell protectants i.e. containing only broth were maintained as check. The formulated liquid biofertilizers of *Azotobacter* were stored in BOD incubator at 28±2 °C for a period of 15 days to see the effect of cell protectants on the mass multiplication of cells. The colony forming units (CFU) were calculated on 0th day and on 15th day. Liquid *Azotobacter* bioinoculants formulated with glycerol (1%) + PEG (2%) promoted long term survival followed by gum arabic (0.3%), PEG (2%) and glycerol (1%) + PVA (0.5%). The results of the present study clearly indicated that the liquid formulation of *Azotobacter* could be used more effectively to maintain the cells viability for long time.

Keywords: Biofertilizer, Shelf life, Bioinoculants, Formulation

1. INTRODUCTION

Biofertilizers are described as group of selective microorganisms like bacteria, fungi and algae, having the capability of fixing atmospheric nitrogen, or which can convert insoluble forms of phosphorus and potassium into soluble forms, and makes it available to plants. They increase the soil fertility. Due to the hazardous effects of chemical fertilizers to environment, plants, animals and human life, there arose a need to develop an ecofriendly, cost-effective and sustainable alternative. So, biofertilizers were introduced to overcome the side-effects of chemical fertilizers and to develop organic farming.

Microbial inoculant technology has emerged as one of the potential tool to enhance the productivity of agricultural systems in the long run. This technology is designed with the principles of sustainable agriculture as opposed to increased use of pesticides and fertilizers. Many bacterial inoculants are exploited to make their use in biofertilizers. *Azotobacter* is one such plant growth promoter that fixes atmospheric nitrogen, produces indole acetic acid, cytokinins, and gibberellin-like compounds. *Azotobacter* belongs to family *Azotobacteriaceae*. It is an aerobic, free-living and non-symbiotic bacteria, which can increase the crop yield by 50%. It can fulfill 80-90% of nitrogen requirement of plant. They are mainly found in the

rhizosphere of rice, bajra, maize, sugarcane and in several vegetable crops [1].

The process of biofertilizer production involves selection of effective microorganisms, its mass multiplication in various size fermenters and then its formulation. Formulation is one of the major step for biofertilizer production, as it behaves as a delivery agent which transports the microbes to the target plant or soil. There are different types of formulations, depending on the carrier, whether it is solid or liquid. Traditionally, talc, which is a clay mineral also known as talcum powder, and peat, which is composed of partially decayed organic matter, were used widely as solid carrier based inoculants. They increase the viability of microbes and protects them from desiccation and harsh environment. Peat have high water holding capacity and large surface area, however it is not available in many countries and also it will be depleted in near future [2]. Also, in the talc based formulations, problems of blockage of nozzles in microirrigation techniques was largely seen. In India, generally lignite, coal or charcoal based bioformulations are used. The major disadvantage associated with thesetype of formulations are shorter shelf life, poor quality, high contamination and unpredictable field experience. Also, the

cost of solid carrier based inoculant production is high as it requires high labor and energy intensive processes such as milling, sieving and correcting pH [3]. The development of liquid bioformulation has several advantages over solid carrier-based formulation like high cell count, less chances of contamination, longer shelf life, greater protection against environmental stresses and increased field efficacy [4]. However, the shelf life of bacteria depends upon bacterial genera, carriers and their particle size [5]. In liquid formulation, the microbial strains are provided with cell protectants like glycerol, polyvinyl pyrrolidone (PVP), polyvinylalcohol (PVA), polyethylene glycol (PEG), Gum Arabic, Trehalose, Sodium alginate, etc., which increases the viability of microbes and helps them to form spores or cysts, offering resistance to abiotic stress. These cell protectants improves the shelf-life of microbes. Thus, the selection of suitable cell protectant is very important.

Considering the above points, in the present study, an experiment was conducted to increase the survival of liquid formulations of *Azotobacter chroococcum* bioinoculants by the addition of different cell protectants- PVA, PEG, Glycerol and Gum Arabic.

2. MATERIALS AND METHOD

Source of strain

Azotobacter chroococcum culture was provided by theVise Organic Innovative Solution Enterprise Pvt. Ltd., Manjusar, Vadodara,Gujarat.

Materials used for preparing liquid formulation

For culturing *Azotobacter chroococcum*, Ashby's and Jensen's N- free media were used. The cell protectants used in formulation were Glycerol, PVA, PEG and Gum Arabic. For enumerating microbial population, nutrient agar was used.

Formulation of liquid biofertilizer inoculants

The *Azotobacter chroococcum* culture obtained from Vise Organic Ltd. was inoculated into Ashby and Jensen's agar plates. The plates were incubated for seven days at $28 \pm 2^\circ\text{C}$ in BOD incubator.

In the following study, there were eight liquid biofertilizer formulations made: B₁- Ashby's N-free broth, B₂-Jensen's N-free broth, B₃-Ashby broth + glycerol (1%), B₄- Ashby broth + gum arabic (0.3%), B₅- Ashby broth + PEG (2%), B₆-Ashby broth + PVA (0.5%), B₇-Ashby broth + glycerol (1%) + PEG (2%) and B₈-Ashby broth + glycerol (1%) + PVA (0.5%). Out of which four formulations i.e. B₃, B₄, B₅ and B₆ were prepared using cell protectants in optimum concentrations. Two broths treatment B₁ and B₂ were maintained without the addition of cell protectants. The

remaining two broths B₇ and B₈, contained a combination of cell protectants.

The sterilized broths with and without cell protectants were inoculated with the *Azotobacter* strain, grown on plates. The cell protectants viz., Glycerol (1%), PVA (0.5%), PEG (2%) and Gum Arabic (0.3%) were added to the broths during the preparation of media only. The inoculated broths were incubated at 28 ± 2°C.

Shelf life study of liquid biofertilizer inoculants

The liquid inoculant formulation, packed in high density polyethylene (HDPE) bottles, were accessed for their shelf life on 0th day (day of inoculation) and on 15th day. The nutrient media was used for enumerating the viable cell population. The serial dilutions were prepared from each formulation up to 10⁻¹⁰. The plates were spreaded with dilutions and incubated at 28 ± 2°C without any disturbance in BOD incubator.

3. RESULTS AND DISCUSSION

To increase the shelf life of *Azotobacter* cells in liquid bioinoculation, the chemicals such as PVA, PEG, Glycerol and GA were added as supplements to Ashby broth.

Survival of liquid bioinoculants

Liquid formulation of *Azotobacter*, was developed in Ashby's Nitrogen free broth added with PEG (2%), glycerol (1%), gum arabic (0.3%) and PVA (0.5%) separately and a combination of glycerol (1%) + PEG

(2%) and glycerol (1%) + PVA (0.5%) were used (**Figure 1, 2**). The results are clearly indicated in **Table 1**. Among the amendments, PEG supported highest number of *Azotobacter chroococcum* cells throughout the observation period followed by gum arabic, PVA and glycerol. The lowest microbial cell count was seen in the broth without cell protectants i.e. Ashby and Jensen's broth. Thus, the cell protectants seemed to increase the mass multiplication of *Azotobacter chroococcum* cells.

Polyethylene glycol (PEG), a low molecular weight (3000), is soluble in water with adhesive and sticky consistency. The adhesive property of PEG enhances cell adherence to seed, and its viscous nature slowsdown the drying process of the inoculant [6]. Gum Arabic, a high molecular weight biopolymer, protects the cells against toxic seed coat factors. It acts as an adhesive, an emulsifier and possess stabilization property which limits heat transfer. It has high water activity [7]. Kumaresan G. et al. (2019), developed liquid formulation for *Pseudomonas fluorescens* amended with trehalose, glycerol and PVP in King'B broth and reported 10⁸ cells/ml up to 10 months storage under room temperature [8]. Singleton P.W et al. (2002), prepared the liquid formulations of *Rhizobium* by adding various additives in the yeast extract

mannitol media and reported cell numbers of 1×10^{10} cells/ml in the liquid inoculant [9]. Enhanced survival of *Azotobacter* cells in the liquid formulation is due to the action of chemical amendments added in the medium. PVA have polymeric properties. It

is known as colloidal stabilization. The improvement of survival is analogous to the protective colloid effect where bacteria represent one colloid and the suspension the other [10].



Figure 1: *Azotobacter chroococcum* grown on Ashby's Nitrogen free plate after 7 days of incubation

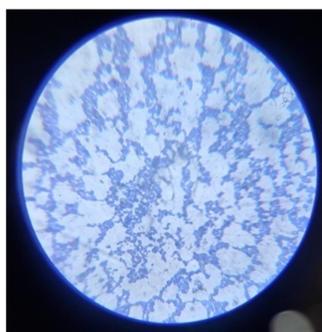


Figure 2: *Azotobacter chroococcum*'s methylene blue stained image under microscope

Table 1: Effect of cell protectants on survival of liquid bioinoculant of *Azotobacter* (Population density ($\times 10^{10}$ CFU/ml or g))

Formulation	0 th day	15 th day
B ₁	21.9	16.2
B ₂	23.7	15.6
B ₃	21.5	19.0
B ₄	26.2	23.4
B ₅	26.0	22.2
B ₆	20.1	19.6
B ₇	27.5	24.0
B ₈	24.2	20.7

Note: B₁: Ashby's N- free broth; B₂: Jensen N-free broth; B₃: Ashby broth + Glycerol (1%); B₄: Ashby broth + Gum Arabic (0.3%); B₅: Ashby + Polyethylene glycol (PEG, 2%); B₆: Ashby broth + Polyvinyl alcohol (0.5%); B₇: Ashby broth + Glycerol (1%) + PEG (2%); B₈: Ashby broth + Glycerol (1%) + PVA (0.5%)

4. CONCLUSION

The liquid bioinoculant formulation can be produced by simple fermentation process

with minimum labour, space and energy, as the culture from the fermentor is directly packed under aseptic conditions and stored.

The cost of production of liquid formulation could be lesser than that of any other type of formulation. From this study, it has been concluded that liquid formulation of *Azotobacter chroococcum* bioinoculants shows that cell protectants maintains the cell viability. Among the different chemical additives PEG (2%) performed well in liquid formulation of *Azotobacter* and hence it can be used in the formulation of liquid bioinoculant.

5. REFERENCES

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