Development of Cost-effective Bio-inoculant Consortium Formulation for Agricultural Usage

Pelapudi Pitchaiah and Ganti Swarnabala

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 06

Res. Jr. of Agril. Sci. (2022) 13: 1762–1764

CARAS

Res. Jr. of Agril. Sci. (Nov-Dec 2022)
13(6): 1762–1764

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

www.rjas.org

Short Communication

Development of Cost-effective Bio-inoculant Consortium Formulation for Agricultural Usage

Pelapudi Pitchaiah[1-2] and Ganti Swarnabala*[3]

Received: 26 Aug 2022 | Revised accepted: 28 Oct 2022 | Published online: 25 Nov 2022
© CARAS (Centre for Advanced Research in Agricultural Sciences) 2022

***Key words*:** Bio-inoculant consortium, Cost effective, Formulation, PGPR, Shelf life

Rhizosphere is the soil around the plant roots where microbial processes are highly influenced by the plant root system. Plant growth promoting rhizobacteria (PGPR) are a heterogenous group of beneficial microbes which enhances the plant growth and yield. This plant growth promoting rhizobacteria protects the plants from the plant pathogens, biotic and abiotic stress conditions. These microbes promote the growth of the plant by supplying essential nutrients by fixing the atmospheric nitrogen and solubilization of phosphorous, potash and zinc [1].

Though there are several notified microbial inoculants viz. *Azotobacter* spp., *Azospirillum* spp., *Pseudomonas* spp., *Rhizobium* spp. available in the market, several drawbacks of the existing microbial inoculants are observed. Most of the market available individual microbial inoculants are inconsistent, low performance in various agroclimatic conditions has brought the consortia-based plant growth promoting rhizobacteria into the spot light [2]. Among all the microbial inoculants, *Bacillus* spp. possess a remarkable protection ability by forming the endospores. These microbes can withstand and survive in harsh environmental conditions and can exhibit the plant growth [3].

Consortium of beneficial microbial inoculants show better efficacy as the bio inoculant formulation are stable in its performances by showing beneficial effects on plants as it broadens the action of its formulation. Various conventional carriers viz. vermiculite, aluminium silicate, talc and bentonite are used for the powder formulations of bio inoculants. These carriers possess various drawbacks *i.e.*, contamination and low shelf life of the product. Similarly, several liquid formulations are available in the market with notified drawbacks like bloating and low shelf life of the product [4]. The present study focuses on developing a cost-effective novel formulation for the *Bacillus* spp. based microbial inoculant with better shelf life.

Collection of plant growth promoting rhizobacteria

The plant growth promoting rhizobacterial strains (*Paenibacillus durus*, *Paenibacillus glucanolyticus* and *Bacillus megaterium*) were collected from the R&D laboratory of Shivashakti Bio Planttec Ltd, Hyderabad.

Development of inoculum

Paenibacillus durus, *Paenibacillus glucanolyticus* and *Bacillus megaterium* were inoculated into the selective medium with composition (g/L) Sucrose 20, K_2HPO_4 0.2, KH_2PO_4 0.4, NaCl 0.3, $MgSO_4$ 0.5, $MnSO_4$ 0.1, $FeCl_3$ 0.01, Na_2MoO_4 0.002, Mica 1, yeast extract 1.5, $Ca_3(PO)_4$ 5, $CaCO_3$ 0.01. The inoculated flasks were kept on the orbital shaker at 120 rpm for 48 h. After 48 h, broth sample was collected and the microbial enumeration was carried out by using the standard spread plate technique [5]. Liquid broth samples were centrifuged (Remi R-4C) at 10000 rpm, cell pellet was collected and also enumerated to assess the microbial count.

Formulation of bioinoculant microbial consortium

2 g of pre gelatinized starch was added to 100 ml of distilled water and boiled till dissolution. 4 g of sodium alginate and 1 g of inulin were added to the starch solution and sterilized. 1 g of cell pellet was added to the above mixture and kept on orbital shaker for 15 min at 120 rpm. Sterilized 100 ml of flax seed oil along with 0.01% Tween 80 was added to the above formulated mixture and kept on orbital shaker for 15 min at 120 rpm. To the formulated product, 1 g of sodium benzoate was added to avoid contamination. Formulated sample was collected and enumeration of microbes was carried out [2].

Shelf-life study of the formulated product

The formulated product was kept in 3 different temperatures i.e., 4 ºC, 37 ºC and 45 ºC to perform the shelf life studies. Samples were drawn from the formulated product every month and the enumeration of microbes were carried out as per

* **Ganti Swarnabala**
✉ swarnabalaganti@gmail.com

[1] Shivashakti Bio Planttec Ltd, S.R. Nagar, Hyderabad - 500 038, Telangana, India

[2] Department of Biotechnology, Jawaharlal Nehru Technological University, Hyderabad - 500 085, Telangana, India

[3] Swaram Biochem, Secunderabad - 500 011, Telangana, India

the standard spread plate technique. Shelf-life studies of the formulated product was carried out for 2 years and the data was recorded.

Vigorous usage of chemical fertilizers and pesticides led to the exploitation of the agricultural lands, human health and environment. In the recent years biofertilizers and biopesticides became popular with their beneficial applications [4]. Usage of plant growth promoting rhizobacterial inoculants is centuries old and yet, further improvements are to be attributed. Though there are several notified benefits of the microbial inoculants, the product inconsistency, low microbial load and delayed results lead to the failure of these products in the market [6]. The drawback lies in the formulation of the products. Though there are various powder and liquid formulations available in the market, many of them failed in protecting the microbes and giving the best shelf life [7]. The present study aims in developing a cost-effective formulation of bio-inoculants and investigating its shelf life.

Potent plant growth promoting microbial strains viz. *Paenibacillus durus* (Nitrogen fixing bacteria), *Paenibacillus glucanolyticus* (Potash solubilizing bacteria) and *Bacillus megaterium* (Phosphate solubilizing bacteria) were collected from R&D laboratory of Shivashakti Bio Planttec Ltd. All the three-plant growth promoting microbial strains were inoculated in the selective media. Final liquid broth and cell pellet collected after 48 h were enumerated by using spread plate technique which showed $1.5x10^9$/ml (final broth) and $2.45x10^{11}$/g (cell pellet).

1 g of cell pellet having $2.45x10^{11}$/g was encapsulated by using the sodium alginate and flax seed oil. Sodium alginate is a natural biopolymer encapsulates the microbial cells and protects them from the harsh environmental conditions. These encapsulated microbial cells will be released by natural depolymerisation mechanism. Inulin present in the formulation acts as a prebiotic and supplies immediate essential nutrients to the microbes in the field condition [2], [8]. The formulated product was tested for its shelf life in three different storage temperatures i.e., 37 ºC (room temperature), 4 ºC (cold temperature) and 45 ºC (elevated temperature). Shelf-life studies at 37 ºC, showed 2.6×10^8/ml after 24 months from 1.2×10^9/ml (Fig 1).

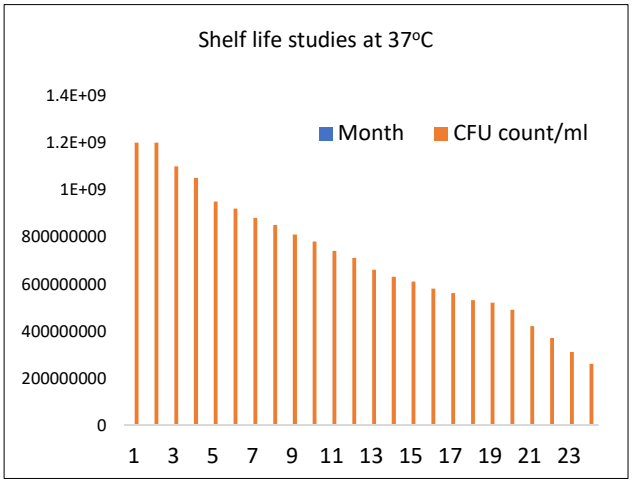

Fig 1 Shelf-life studies of the bio-inoculants at 37 ºC

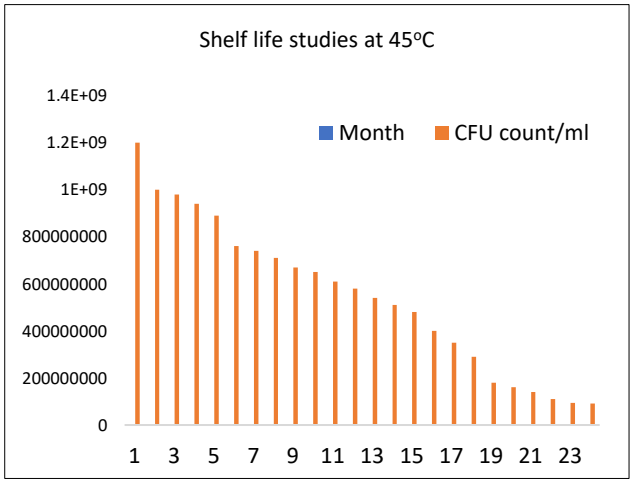

Fig 2 Shelf-life studies of the bio-inoculants at 45 ºC

Shelf-life studies were carried out at 45 ºC, a drop in the microbial count from 1.2×10^9/ml to 9.1×10^7/ml was noticed. This is due to the loss of vegetative cells in the elevated temperatures (Fig 2).

Product shelf-life studies conducted at 4ºC showed slight drop in the microbial counts from 1.2×10^9/ml to 6.7×10^8/ml (Fig 3). When compared to room temperature and elevated temperatures, very less microbial loss was recorded at 4 ºC. In cold conditions, metabolic activities rate of the microbes will be very less because of which the microbes will remain under static conditions without losing its viability.

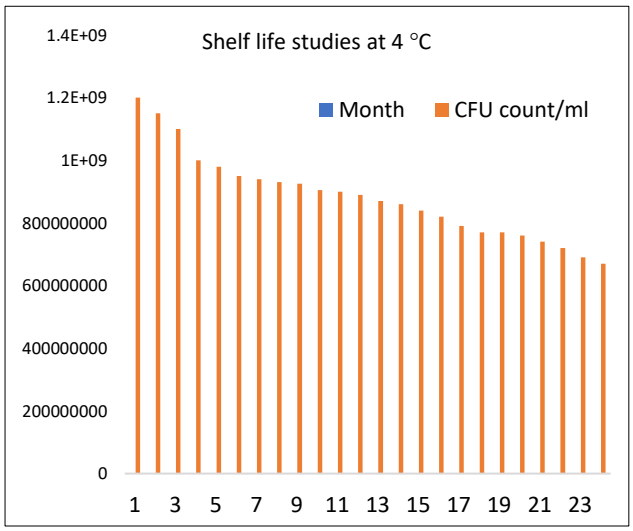

Fig 3 Shelf-life studies of the bio-inoculants at 4 ºC

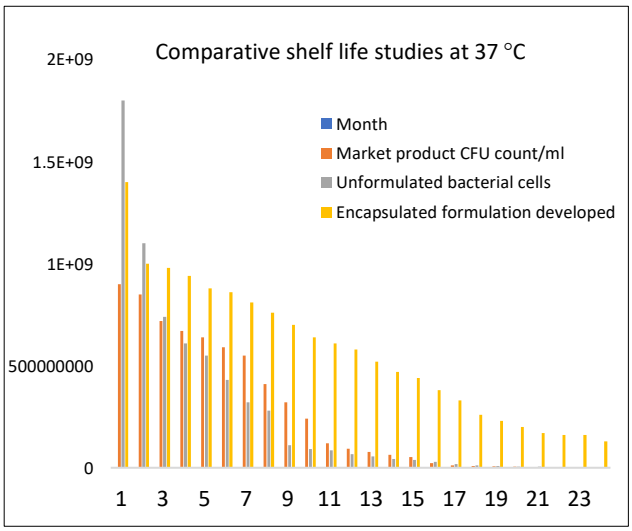

Fig 4 Comparative shelf-life studies at 37 ºC

Comparative shelf-life studies were carried out by taking the market proven plant growth promoting consortium-based product, unformulated microbial cells and developed encapsulated formulation. Results showed high stability in the developed product formulation when compared to the unformulated and market product. The microbial loss in the developed formulation is 1.3×10^8/ml from 1.4×10^9/ml (Fig 4).

SUMMARY

From the last decade, awareness on usage of plant growth promoting rhizobacteria (PGPR) is increasing on a rapid scale. Though several advantages of PGPR were noticed, several disadvantage of individual microbial inoculants viz. inconsistency, poor performance in various geographical locations were noticed. The present research aims in developing a cost effective PGPR consortia-based formulation with high stability. The formulation developed showed high stability at 37 ^oC (2.6×10^8/ml), 45 ^oC (9.1×10^7/ml) and 4 ^oC (6.7×10^8/ml). Shelf-life comparison study with the market product and unformulated microbial cells showed high stability in the developed encapsulated liquid formulation (1.3×10^8/ml). Based on the results, it can be concluded that the developed formulation can be effectively used for the formulation of PGPR consortium. The present research work targets, cost effective plant growth promoting consortium-based formulation with better shelf life. Based on the above results, it can be concluded that the developed encapsulated liquid formulation showed high stability when compared to the market product and the unformulated microbial cells. So, the developed formulation can be effectively used for the formulation of plant growth promoting microbial consortium.

Conflict of interest

Authors have no conflict of interests.

LITERATURE CITED

1. Rocheli DS, Adriana A, Luciane MPP. 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and Molecular Biology* 38(4): 401-419.
2. Venkata RN, Karuganti S, Babul RG, Manasa B, Venu P, Praveena K. 2020. Usage of Encapsulated Plant Growth Promoting-Microbial Consortia and Testing its Efficacy on *Cajanus Cajan*. *Journal of Microbiology and Biotechmical Technology* 12(7): 1-4.
3. Sandra SS, Ivana K, Vladimir B, Nada N, Miodrag N. 2019. Bacillus based microbial formulations: Optimization of the production process. *Hemijska Industrija* 73(3) 169-182.
4. Vinod K, Sarma MVRK, Krishna S, Rashmi S, Lalit K, Vikram S, Bisaria VS, Sharma AK. 2012. Effect of formulated root endophytic fungus *Piriformospora indica* and plant growth promoting rhizobacteria fluorescent pseudomonads R62 and R81 on *Vigna mungo*. *World Journal of Microbiology and Biotechnology* 28: 595-603.
5. Pelapudi P, Sasikala C, Swarnabala G. 2021. Isolation, identification and characterization of native plant growth promoting bacteria and their plant growth promotion in *Zea mays*. *Research Journal of Biotechnology* 16(8): 75-80.
6. Yoav B, Luz EDB, Prabhu SR, Juan PH. 2014. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant Soil* 378: 1-33.
7. Denaya S, Yulianti R, Pambudi A, Effendi Y. 2021. Novel microbial consortium formulation as plant growth promoting bacteria (PGPB) agent. *IOP Conf. Series: Earth and Environmental Science* 637: doi:10.1088/1755-1315/637/1/012030
8. Woo SL, Pepe O. 2018. Microbial consortia: Promising probiotics as plant biostimulants for sustainable agriculture. *Opionion* 9: 1801. doi: 10.3389/fpls.2018.01801.