

# Bio Formulation for Cicer Arietinum L's Nutrient Enhancement that includes Cohorts of Ensifer Adhaerens MSN12 and Bacillus Cereus MEN8

## Abstract

Ensifer adhaerens MSN12 and Bacillus cereus MEN8, two taxonomically distinct entophytic bacteria, form a group with promising plant growth promoting (PGP) properties. Through the creation of an efficient bio inoculant with cost-effective and readily available carriers, we demonstrate their transfer from the laboratory to the field. It was discovered that sugarcane straw ash (SCSA) is an effective carrier and bio formulation for enhancing strain viability and shelf life of up to one year. Seed germination, plant weight, length, seed yield, harvesting index, and proximate nutritional constituents were all increased by 20% over consortium treatment without SCSA in a bio formulation containing an SCSA-based consortium (MSN12 + MEN8). In addition, the bio formulation of post-harvest treated soil outperformed a pre-sowing SCSA-based bio formulation treated crop in terms of its physicochemical properties. The soil was enriched in a variety of proximate nutritional constituents, including dry matter (30 percent), crude protein (45 percent), crude fiber (35 percent), and ether extract (40 percent) in comparison to the control. A positive correlation between the treatments was found in scattered matrix plots and principal component analysis, confirming the T6 treatment's improvement in soil nutrient components and proximate constituents (MSN12 + MEN8 + SCSA). For adequate delivery of laboratory strains as a substitute for chemi-fertilizers, the aforementioned results suggest that SCSA is effective not only as a carrier material but also as a support for microbial growth.

**Keywords:** Endophyte • Bacterial consortium • Bio formulation • Sugarcane straw ash (SCSA) • Soil fertility • Plant growth

## Introduction

However, the texture and nutrient yield of produce, soil quality, native micro flora, and consumer health are all impacted by the long-term use of agrochemicals. As a result, a healthy alternative to chemical fertilizers that can encourage plant growth is urgently required. On the other hand, the lack of field trials, decreased food security, and ecological sustainability have opened up new possibilities for rethinking how microbial inoculants are applied using the available carrier materials. For the formulation of bio-inoculants, which have the potential to support the growth of microbial strains, the selection of low-cost carriers is still in its infancy. Additionally, the selected microbial strains must have a distinct dominance in the rhizosphere to support plant growth and development. Bio inoculants have recently been made with a variety of known carrier materials made from various microorganisms. In fact, bio inoculants are an environmentally friendly and financially viable alternative to bacterial delivery in agriculture that has not yet made it into the agro-market. Agrochemical use, on the other hand, has a negative impact on soil nutrient status and microbial diversity. Therefore, it is essential to focus on beneficial microorganisms in order to develop environmentally friendly methods of soil enrichment that do not harm the environment or reduce microbial diversity. Plant health, biomass, and yield can all be improved through interactions with beneficial bacteria. Local carrier materials that are suitable for as many bacterial species and strains as possible are used in a formulation of bio inoculants. The appropriate

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supporting material needs to be safe, non-toxic, and long-lasting. For agricultural products, storage temperature and moisture content are crucial factors. As a result, new approaches to transporting beneficial microorganisms from the laboratory to the real world need to be considered. These new approaches may prove to be more beneficial [1-5].

## Materials and Method

### Root-Nodulating and Non-Nodulating Endophytic Bacteria Isolation and Characterization

The chickpea plant's 2-centimeter-long root nodules and root pieces were used to wash and rinse adherent rhizospheric soil. Following the serial dilution method, the macerated nodules and root segments were plated on Yeast Extract Mannitol Agar (YEMA) and Luria-Bertani (LB) Agar media, respectively. The Petri-plates were then incubated at 30 °C for 24 to 48 hours until the bacterial colonies on the plate began to grow. In order to search for various morpho-types, the bacterial colonies that had grown were purified through successive culturing. A field-first approach was used to select beneficial bacterial strains.

### Bacterial Consortium and Synergistic Interaction

Following the standard protocols of Pierson and Weller and Pandey and Maheshwari, the most suitable strain with PGP characteristics was chosen for the consortia and grown together to determine its efficacy. After each of the nine strains had grown independently in the nutrient broth, 100 L of each culture was spread out on the nutrient agar media (NAM) plates, incubated at 28 °C for 24 to 48 hours, and the isolates were restreaked for purification following their growth. However, a single colony of MEN8 and MSN12 was transferred into the fresh nutrient broth for the bacterial consortium and incubated at 28 °C on the rotatory shaker at 120 rpm. Using a UV-Vis Spectrophotometer, the absorbance at a wavelength of 600 nm was measured to determine additional growth. In order to grow the consortium culture, 100 mL of fresh nutrient broth was added to 100 mL of active cultures of MEN8 and MSN12 with an OD of 0.6 at 600 nm.

## Discussion

Due to their superior colonization efficacy and capacity to alleviate biotic and abiotic stresses,

endophytic microorganisms are currently gaining popularity in sustainable agriculture. However, it is still challenging to select suitable supporting materials for the preparation of bacterial inoculants. As a result, one way to increase crop productivity is to evaluate inoculated bacteria in supporting materials. PGP *Ensifer adhaerens* MSN12 and *Bacillus cereus* MEN8 demonstrated their inherent capacity to synthesize IAA in this study. IAA production was previously reported to be supported by other bacterial genera. Reported rhizospheric isolates that have the potential to produce IAA. When applied, these isolates significantly increased plant growth while also maintaining the quality of the nutrients in the soil. In our research, both MSN12 and MEN8 solubilized phosphate (P) and potassium (K) salts to make them more readily available to the chickpea plant. As can be seen, various mechanisms for rhizospheric *Ensifer* and *Bacillus* strains' solubilization of insoluble P in the soil have been developed. Additionally, the MSN12 and MEN8 strains produced siderophores, each of which helps the chickpea grow [5-10].

## Conclusions

There must be a sustainable farming method recognized in light of the current climate change situation. Utilizing microbial consortia is an emerging strategy for increasing agricultural yield in this setting. SCSA significantly ( $p < 0.05$ ) increased shelf life and supported the growth of *E. adhaerens* MSN12 and *B. cereus* MEN8 strains in our study. In terms of plant growth, the responses of the two strains were favourable. Seed germination, plant growth, yield, and soil fertility were significantly improved by the bio formulation or consortium of SCSA, *E. adhaerens* MSN12, and *B. cereus* MEN8. As a result, the tested bio formulation has the potential to increase agricultural yields and can be utilized industrially to deliver a sufficient number of microbes for chickpea cultivation by substituting chemical fertilization for it.

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