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Increasing Efficiency of Liquid Fertilizer via Incorporating Beneficial Microorganisms

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ABSTRACT

The growing trend in organic agriculture has boosted the public awareness of organic fertilizer. The present study focused on isolating plant growth promoting microorganisms from the soil samples and incorporating beneficial plant growth promoting microbial (PGPM) strains to a provided liquid organic fertilizer to improve the efficiency of current formula. After isolating plant growth promoting microorganisms, experiments were conducted qualitatively and quantitatively to evaluate the efficacy of those species. Five phosphorous solubilizing bacteria and fungi, one potassium solubilizing bacteria, one potassium solubilizing fungi, six free living nitrogen fixing bacteria from different regions including Hambanthota, Mahiyanganaya, Galaha, Welimada, Rathnapura Sri Lanka were isolated using serial dilution plating on specific growth media and screened for various plant growth-promoting traits. The highest phosphate solubilization (67.8 mg/ml) was exhibited in PH.1 which also exhibited the highest phosphorous solubilization index (PSI) of 2, isolated from the soil sample received from Hambanthota district. Alginate encapsulation as small beads were produced from bacterial inoculum of PH.1 phosphorous solubilizing bacteria with sodium alginate, cellulose, and

calcium chloride. A series of different percentages of cellulose (3% - 6%) was used during bead formation to evaluate the effect of cellulose on encapsulation efficiency of beads. Alginate beads were applied to the liquid fertilizer, incubated, and plated periodically to evaluate the efficiency of this formulation. The number of released cells of PH.1 reached 7.36 X 10 6 CFU/ml after 48 hours of incubation in the 0.25 X diluted liquid fertilizer which resulted from the bead formulation of 4% (w/v) Alginate + 3% (w/v) cellulose. The cellulose supported the entrapment of bacterial cells (plant growth-promoting bacterium) PH.1 as biofertilizer in the matrix, which reduced cell loss. The highest entrapment efficiency of 5.441% was obtained at 3% (w/v) cellulose, Overall, the appropriate content of cellulose mixed with alginate is conducive to changes in the morphology of microcapsules and increases the amount of biological encapsulation. This indicates that the beads-based biofertilizer can partially replace chemical fertilizers.

1. INTRODUCTION

Fertilizer is required to provide nutrients to the soil, which promotes plant development and increases agricultural yield. However, long-term use of these inorganic fertilizers affects both food quality and eco-systems. As a result, new environmentally friendly fertilizers, such as bio fertilizers, have been developed to partially replace inorganic fertilizers. Beneficial microorganisms may easily infiltrate the rhizosphere after being applied to the soil and provide nutrients to plants. With all the limitations of chemical fertilizer, using microorganisms capable of converting nutrients that the soil contains into forms that may be readily taken by plants has received attention (Szopa et al., 2022). The objectives of the present study were to isolate phosphate solubilizing, potassium solubilizing, and nitrogen fixing microbial strains, assess their plant growth-promoting characteristics and incorporate the most efficient strain to develop organic liquid fertilizer using a microbial carrier. Due to their quantity, availability, and low cost of peat, clay, and bentonite, they are the most utilized carriers. Although because of their high nutrient content, these materials are easily contaminated. (Schoebitz et al., 2013) As a result, polymeric-based carriers for the manufacturing of bio fertilizers have been created, as these organic molecules can be used as a carbon source for bacteria, so extending their survival during storage. Moreover, polymer molecules can be cross-linked to produce three-dimensional structures that protect bio fertilizers from contamination. Because of their biodegradability and compatibility, alginate gels are commonly employed as polymer carriers. Alginate is the most used polymer substance for encapsulating microorganisms for a variety of industrial microbiological applications (Bashan et al., 2002). Bacteria can integrate into the gel in the wet or concentrated dry beads after inoculation, making them easy to store, transport, and apply. Unfortunately, as alginate solution is exceedingly viscous and weak, these alginate beads are nonuniform, very porous, and have poor mechanical stability. Fillers like starch can be added to the formulation to increase the dry matter in the beads, which improves mechanical resistance and allows for the slow release of beneficial bacteria (Rohman et al., 2021). Additional matrix-forming components not only increase the mechanical characteristics, swelling qualities, and stability of the capsules, but they also boost bacteria encapsulation efficiency (Szopa et al., 2022). Furthermore, the addition of cellulose boosts the mechanical strength of alginate beads and improves bacterial survivability during the manufacturing, drying, and storage processes. The current study indicates an improvement in the encapsulation of plant growth promoting rhizobacteria utilizing alginate as a carrier by supplementing with cellulose to support bacterial viability. The capability of bacteria to release into liquid fertilizer from beads with varying percentages of additives (cellulose) was also evaluated.

2. MATERIALS AND METHODS

2.1. ISOLATION OF EFFICIENT MICROBIAL STRAINS

Rhizosphere soil samples collected from agricultural lands in three different zones: wet zone, intermediate zone, and dry zone of Sri Lanka were employed in isolating nitrogen fixing, phosphate and potassium solubilizing, bacterial and fungal strains. Each soil sample were serially diluted and cultured on the specific media such as NBRIP medium for phosphorous solubilizing microorganisms, Alexandrow medium for potassium solubilizing microorganisms and N free medium for nitrogen fixing bacteria isolation of plant growth promoting microorganisms.

2.2. MICROSCOPIC OBSERVATIONS AND MOLECULAR IDENTIFICATION OF ISOLATED MICROBIAL STRAINS

Morphological differences of each isolated strains were identified by compound light microscope and the observations were recorded at 600X magnification. For the identification of species, genomic DNA was extracted from each isolated strain and DNA products were used for Polymerase Chain Reaction to amplify the V4 region of 16s rRNA gene of bacteria and ITS region of fungi. With agarose gel electrophoresis, PCR products were visualized.

2.3. EFFICACY TESTS

Qualitative screening was conducted for phosphorous solubilizing microorganisms and potassium solubilizing microorganisms by measuring the Phosphorous solubilizing index (PSI) and potassium solubilizing index (KSI). Quantitative screening was conducted for phosphorous solubilizing bacteria by colorimetric determination of soluble phosphorous of microbial cultures by Olsen test. (*METHODS OF SOIL ANALYSIS Part 2, Second Edition*, 1982). As a quantitative screening of nitrogen fixing bacteria, the efficiency of nitrogen fixing ability of the isolates were determined by Khjeldal analysis as described by (Patrick et al., 2018) inoculating the isolates in nitrogen free Jenson's broth medium which contains sucrose as carbon source and incubated at 32°C (Samarathunga & Chandrasena, n.d. ; Panneerselvam, 2010). The values were means of at least two replications.

2.4. ENCAPSULATION OF PLANT GROWTH-PROMOTING BACTERIA IN ALGINATE BEADS ENRICHED WITH CELLULOSE.

2.4.1. BEAD FORMATION

Phosphorous solubilizing bacteria encapsulated cellulose-alginate beads were prepared by extrusion encapsulation method (Bashan et al., 2002). The cellulose content of dispersions varied at 3, 4, 5 and 6% (w/v). To obtain bead matrix solutions for alginate / cellulose blends, 4% (w/v) alginate was added into each dispersion. Calcium chloride solution was used as the crosslinking agent.

2.4.2. DETERMINATION OF THE RELEASING ABILITY OF BACTERIA FROM THE BEAD TO DIFFERENT CONCENTRATION OF LIQUID FERTILIZER.

Experiments were set up with encapsulated plant growth promoting bacteria (cellulose and sodium alginate beads) in different liquid fertilizer concentrations to evaluate the release of bacteria from the bead to liquid fertilizer. Sterile liquid fertilizer with [X] concentration and diluted sterile liquid fertilizer with 0.25 X concentration which was diluted into 25 % of the volume from original concentration using sterile distilled water were served as the incubation medium for bacteria encapsulated beads. Sterile distilled water was served as the positive control. From each sample, amount of 20 beads were transferred to 20 ml of sterile distilled water (served as the control), sterile liquid fertilizer with [X] concentration and

0.25X diluted sterile liquid fertilizer were incubated at room temperature and the number of cells released from the bead into respective media was counted at different incubation intervals (24 and 168 hours) by standard plate count method.

3. RESULTS AND DISCUSSION

3.1. MICROSCOPIC OBSERVATIONS OF ISOLATED MICROBIAL STRAINS

A total of 5 bacterial isolates (PH.1, PG.3, PG.4, PM.1, PM.2) with distinctly different morphologies which exhibited clear zones (with the PSI in the range of 1 – 2) and a total of 2 fungal isolates (PG1.2, PM.4) with distinctly different morphologies which exhibited clear zones (with the PSI in the range of 1 – 1.1) around the colony after 7 days of incubation at 30 ^{0}C were selected as phosphate-solubilizing microorganisms. One bacterial isolate (KW.1) and one fungal isolate (KG) with distinctly different morphologies that exhibited clear zones (with the KSI in the range of 1.7 – 2.9) around the colony after 7 days of incubation at 30 ^{0}C were selected as potassium solubilizing microorganisms. Six morphologically distinct nitrogen fixing bacterial species (NG.1, NG.4, NM.1, NM.2, NW.1, NH.1) were isolated.

3.2. EFFICACY TESTS RESULTS

3.2.1. QUALITATIVE SCREENING OF PHOSPHOROUS SOLUBILIZING BACTERIA AND FUNGI.

Table 1: Phosphate solubilization efficiency expressed as phosphate solubilization index (PSI) of bacterial isolates in plate assay in NBRIP agar.

Isolate	Colony diameter (mm)	Colony + halo zone diameter (mm)	PSI
PH.1	3	7	2
PG.3	5	7	1
PG.4	10	11	1.1
PM.1	7	8	1
PM.2	3	4	1
PM.4	44	48	1.1
PG1.2	7	9	1

3.2.2. QUANTITATIVE SCREENING OF PHOSPHOROUS SOLUBILIZING BACTERIA.

Table 2: Inorganic phosphate solubilization efficiency of phosphorous solubilizing bacteria in liquid NBRIP medium during 14 days of incubation.

Isolate	The concentration of soluble phosphorous (ppm) (mg/ml)
PM.2	32.5
PG.4	28.6
PH.1	67.8
PG.3	30
PM.1	60

Encapsulation of Plant Growth-Promoting Bacteria in Alginate Beads Enriched with cellulose. Determination of the releasing ability of bacteria from the bead to different concentration of liquid fertilizer.

With the results of the efficacy tests conducted for phosphorous solubilizing bacteria, the highest phosphate solubilization was exhibited in PH.1. Alginate encapsulation as small beads were produced from bacterial inoculum of PH.1 phosphorous solubilizing bacteria with sodium alginate, cellulose, and calcium chloride.

This experiment used the liquid fertilizer as the same composition available in the market and it was mentioned as “X”. Liquid fertilizer diluted with 25% of sterile distilled water was mentioned as 0.25 X.

Bacteria were released to the water except sterilized liquid fertilizer (both X and 0.25 X) after 24 hours of incubation. After 48 hours of incubation, bacteria were released into water and 0.25 X sterile liquid fertilizer. Encapsulation efficiency was measured in this study by counting the survivors after the release of encapsulated bacterial cells and was expressed as a percentage

efficiency. (Figure 1)

Figure 1: Encapsulation efficiency of alginate beads blended with various contents of cellulose and inoculated with phosphorous solubilizing bacteria.

The significance of these findings was to improve the efficacy of liquid fertilizer including PGPM. That was addressed during the study as the main challenge is applying plant growth promoting microorganisms to plants that encourage growth. When microorganisms are added directly to liquid fertilizer, they are subjected to potentially dangerous environmental conditions like high temperatures and fluctuating pH levels. All of which have the potential to destroy an organism and diminish its ability to benefit crops. Techniques for spreading and immobilizing microorganisms, such as the production of bio encapsulated bacteria, have gained importance to overcome these challenges and increase their effectiveness.

4. CONCLUSION

Biological encapsulation is a promising method of carrying plant growth promoting bacteria for biofertilizer delivery and could be a futuristic solution to the problems presented by conventional carriers. In this work, alginate/ cellulose blends were successfully used to encapsulate Phosphorous solubilizing plant growth-promoting bacteria. The synergism produced by incorporating cellulose into the alginate matrix resulted in an alginate/cellulose network that improved the morphology and encapsulation efficiency of the beads. Cellulose improved the entrapment of bacterial cells in the matrices during encapsulation. The highest encapsulation efficiency was obtained from beads with 3% (w/v) cellulose, in 0.25X diluted liquid organic fertilizer.

As a conclusion, this work is on the encapsulation of Phosphorous solubilizing bacteria for agricultural purposes using alginate/cellulose blend and may be an alternative method to incorporate plant growth promoting microorganisms into fertilizer products which will render easier application to cultivation in the future with further research. To facilitate the faster adoption of this technology, research must be done to determine the optimal bio encapsulation technique that considers the needs of the crop, the microorganism being used, and the specific environmental conditions. For beneficial bacteria to remain viable as bio fertilizers, this approach should help sustain their population and guarantee their efficient dispersion throughout the soil.

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