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Enrichment and Biopelletization of Phosphate Solubilizing Diazotrophic Bacterial Isolates Using Earthworm as a Tool

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Authors' contributions

This work has been carried out by both the authors. Author MC designed the study, performed the statistical analysis, wrote the protocol, and the first draft of the manuscript. Author GVV managed the analyses of the study and literature searches. Both the authors have read and approved the final manuscript.

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ABSTRACT

Aims: The beneficial role of earthworm *Eudrilus eugeniae* in enhancing the populations of two phosphate solubilizing diazotrophic isolates viz., *Bacillus* sp. (DT) and *Azotobacter chroococcum* (DT), isolated from the rhizosphere of finger millet [*Eleusine coracana* (L) Gaertn] was studied.
Place and Duration of Study: The experiments were conducted at the campus of M/s. Chaitra Biofertilizers and Chemicals (P) Ltd, Mysore, between January – June 2018.
Methodology: To the glass jars containing three week old partially decomposed green leafy material and cow dung, 0.5 gram of lignite based phosphate solubilizing diazotrophic isolates were added. To this eight medium sized earthworms were allowed and moisture is maintained at 50-60%. The population of isolates in the gut of earthworm and vermicasts were estimated on 2nd, 20th, 40th and 60th day by dilution plate method using Pikovskaya's and Jensen's agar medium
Results: The population of *Bacillus* sp. (DT) and *A. chroococcum* (DT) increased by 22.14, 42.14 and 97.62 percent in the fore gut, mid gut and hind gut regions respectively while *A. chroococcum*

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(DT) by 24.05, 41.19 and 110.95 percent as compared to their initial population in the feeding material. Both the isolates increased enormously in the vermicasts up to 40th day and thereafter declined as recorded in 60 days old vermicasts. *Bacillus* sp. (DT) increased by 196.91 and 247.16 percent in 20 and 40 day old vermicasts and decreased by 54.72 percent on 60th day while *A. chroococcum* (DT) recorded an increase of 217.34 and 270.77 percent and thereafter declined by 41.18 percent in 60 days old casts.

Conclusion: The earthworm can be used as a tool for secondary level multiplication and biopelletization of the isolates to produce enriched vermicompost for use in finger millet cultivation. It also indicated that the vermicasts should be applied soon after it is harvested.

Keywords: Earthworm; diazotrophs; Bacillus sp.; Azotobacter chroococcum; vermicasts.

1. INTRODUCTION

The importance of earthworms in sustaining soil fertility and productivity is known since 1881 when Darwin published his last scientific book "Formation of vegetable mould through the action of earthworms with observation on their habits". Earthworms play a major role in the soil physical, chemical and biological properties [1]. They accelerate the turnover of soil organic matter and mineralization especially nitrogen mineralization through direct and indirect effects on the microbial community [2]. The interactions between earthworms and microorganisms provide the nutrients and also stimulates the plant growth indirectly [3]. Earthworms and microorganisms have complex interrelationships with microorganisms. The earthworms depend upon microorganisms as their major source of nutrients, they promote microbial activity in decaying organic matter by fragmenting it and inoculating it with microorganisms and they also disperse microorganisms widely through soils [4]. Microflora get influenced by earthworms directly or indirectly through comminution, burrowing, casting, grazing and dispersal which change the physico-chemical and biological status of the soil or substrate which brings shifts in the density, diversity, structure and activity of microbial and faunal communities [5]. Certain microbes may be enhanced, reduced or unaffected depending on their ability to adapt to the earthworm drilosphere. It has been reported that the positive influence of earthworms in increasing the population of two P-solubilizing bacterial inoculants during gut transit and in vermicasts. The present study reports such beneficial effects of earthworms on two P-solubilizisng diazotrophic bacterial isolates from the rhizosphere of finger millet which have been shown to improve grain and straw yield in finger millet [6,7]. The vermicasts represent biopelletized microbes in decomposed organic matter which act as inoculation loci when applied to the soils.

2. MATERIALS AND METHODS

The studies were conducted in glass jars measuring 17 x 8.5 x 25 cm (L x B x H) with a substrate holding capacity of 500 gm. The earthworm *Eudrilus eugeniae* was used. The composting material was prepared by mixing equal quantities of green leafy material and cow dung. The material was allowed for partial decomposition for three weeks. Later, the jars were filled with 500 gm of partially decomposed material. To each jar, 0.5 gm of selected lignite based phosphate solubilizing diazotrophic bacterial isolates *Bacillus* sp. (DT) and *A. chroococcum* (DT) were added separately and mixed thoroughly. Eight medium sized earthworms were allowed in to each jar for worm working. Moisture was maintained at 50-60 percent throughout the period of study. The inoculant population in the gut of earthworm and in vermicasts was estimated by dilution plate method using Pikovskaya's and Jensen's agar medium. Two sets of jars were kept for each bacterial isolate, ten replicates each to study the population in the gut and collecting vermicasts on 2nd, 20th, 40th and 60th day. The earthworms in the first set of jars were used to study the phosphate solubilizing diazotrophic bacterial population in the gut. The second set was used to study the inoculant population in the vermicasts.

To study the gut flora, the earthworms from each jar were separately dissected and the fore gut, mid gut and the hind gut regions were removed, weighed and macerated in separate sterile test tubes and appropriate dilutions were prepared in distilled and sterilized water. From appropriate dilutions, 0.1 ml of suspension was plated and spread on Pikovskaya's and Jensen's agar media separately to estimate the population.

The vermicasts were collected on 2nd, 20th, 40th and 60th day from each of the jars maintained for the purpose. One gram of vermicasts were placed in 100 ml distilled and sterilized water and stirred on a magnetic stirrer for 30 minutes. Appropriate dilutions (0.1 ml) were plated separately on Pikovskaya's and Jensen's agar media and spread with a sterile spreader. The plates were incubated at 28-30°C for three days in an incubator and the colony forming units were counted. The population was estimated per gram of the sample. The population increase in the gut regions were estimated against their initial population in the feeding material. The data obtained were statistically analyzed following the method developed by Sundarraj et al. [8].

3. RESULTS AND DISCUSSION

The results on the population of bacterial isolates in the gut and vermicasts are presented in Table 1. The bacterial inoculants were stimulated in the gut and vermicasts recording significant increase in their population. Gradual increase in their population was recorded in the gut with highest population in hindgut region. The population of *Bacillus* sp. (DT) increased by 22.14, 42.14 and 97.62 percent in the fore gut, mid gut and hind gut regions respectively and the corresponding figures for *A. chroococcum* (DT) were 24.05, 41.19 and 110.95 percent as compared to their initial population in the feeding material (4.20 x 10^3 cfu's/gm) (Fig. 1).

Enormous amount of increase in the isolate population was recorded in the vermicasts up to 40th day which later declined significantly in 60th day old casts. However, the population of the isolates under a particular age of vermicasts was on par with each other. *Bacillus* sp. (DT) population was 196.91 and 247.16 percent more on 20th and 40th day as compared to its population in two day old vermicasts. On the 60th day the population decreased by 54.72 percent as compared to its population on

Table 1. Enrichment of vermicompost with phosphate solubilizing diazotrophic bacterial isolates through biopelletization using earthworm as a tool (Average data of four experiments)

Isolate	Population (cfu's/g) x 10^3			Age of vermicasts (in days) and population (cfu's/g) x 10^4			
	Fore gut	Mid gut	Hind gut	2	20	40	60
Bacillus sp. (DT)	5.13	5.97	8.30	10.37 (3.5)	30.79 (6.10)	36.00 (5.17)	16.30 (2.10)
Azotobacter chroococcum (DT)	5.21	5.93	8.86	9.17 (3.9)	29.10 (6.58)	34.00 (5.33)	20 (2.36)
CD @ 5%	3.09	3.80	2.51	3.71	3.52	4.30	2.07

Initial inoculant population: 4.20 x 10^3 cfu's/gm of composting material
Figures in parenthesis are the population in uninoculated control

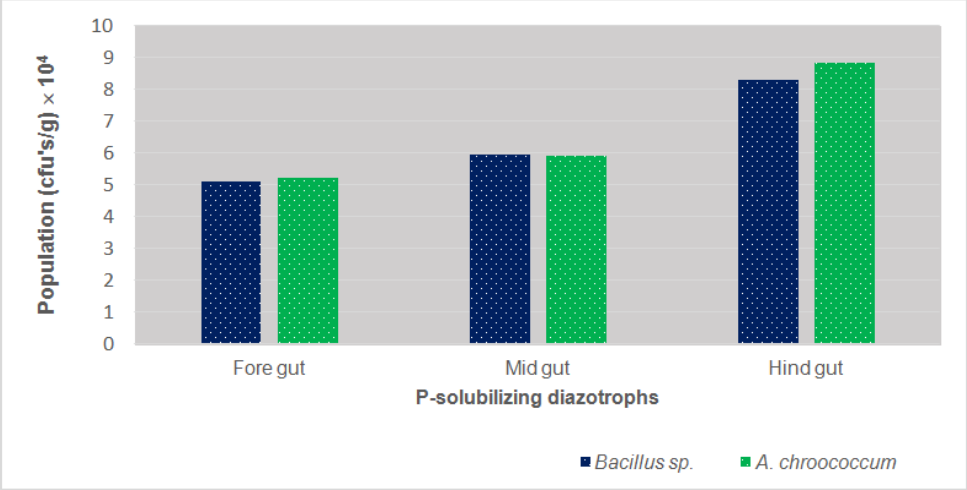

Fig. 1. Population of P-solubilizing diazotrophs in the gut region of *Eudrilus eugeniae*

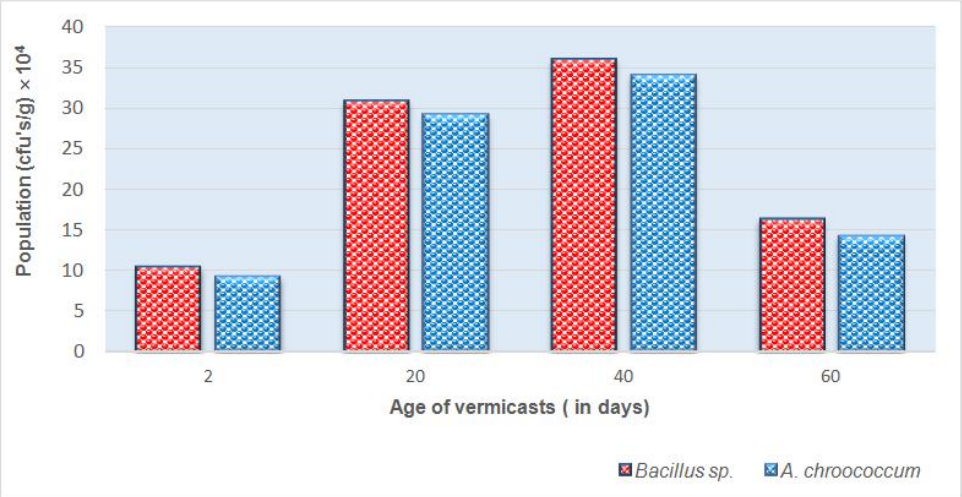

Fig. 2. Population of P-solubilizing isolates in relation to age of vermicasts

40th day. Similarly, *Azotobacter chroococcum* (DT) increased by 217.34 and 270.77 percent on 40th day but declined by 41.18 percent on 60th day as compared to their population on 40th day (Fig. 2).

Significant increase in the total number of bacteria in the gut of earthworm compared to the surrounding soil has been reported [9]. There is an exponential increase in microbial population from anterior to the posterior portions of the earthworm gut [10,11] and great increase in microbial populations in the earthworm casts compared to the surrounding soil and this may be partially due to large amounts of water and mucus that earthworms secrete into their guts [12,13]. The intestinal mucus produced by earthworm contained large amounts of water soluble, low molecular weight organic compounds that could be assimilated easily by rapidly multiplying microbial community in the gut and later in the excreted vermicasts [14]. Vermicasts are usually rich in ammonia and partially digested organic matter and thus provide a good substrate for growth of microorganisms. Some of the intestinal mucus secreted during passage through gut is egested with the casts which are in the form of pellets, where they continue to hold moisture and stimulate microbial activity and growth [14,15]. The results of the present study and the reports of the above workers are in conformity and shows that the earthworms can be exploited to produce enriched vermicompost by the farmers and commercial vermicompost producing companies. However, care should be taken to use the isolates that survive the passage through earthworm gut [16].

4. CONCLUSION

The bacterial inoculants were stimulated in the gut and vermicasts recording significant increase in their population. Gradual increase in their population was recorded in the gut with highest population in hindgut region. The results and the reports are in conformity and shows that the earthworms can be exploited to produce enriched vermicompost by the farmers and commercial vermicompost producing companies. The earthworm can be used as a tool for secondary level multiplication and biopelletization of the isolates to produce enriched vermicompost for use in finger millet cultivation. It also indicated that the vermicasts should be applied soon after it is harvested.

DISCLAIMER

This paper is based on preliminary dataset. Readers are requested to consider this paper as preliminary research article, as authors wanted to publish the initial data as early as possible. Authors are aware that detailed statistical analysis is required to get a scientifically established conclusion. Readers are requested to use the conclusion of this paper judiciously as statistical analysis is absent. Authors also recommend detailed statistical analysis for similar future studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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