

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

Aims: The beneficial role of earthworm *Eudrilus eugeniae* in enhancing the populations of two phosphate solubilizing diazotrophic biofertilizer 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 material of 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 added 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 Jenson'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* (DT) by 21.43, 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 54.7 percent on 60th day while *A. chroococcum* (DT) recorded an increase of 217.14 and 270.11 percent and thereafter declined by 58.24 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; diazotrophic; *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 accelerates the turnover of soil organic matter and mineralization and especially nitrogen mineralization through direct and indirect effects on the microbial community [2]. The interactions between earthworms and microorganisms provides the nutrients and also stimulates the plant growth indirectly [3]. Earthworms and microorganisms have complex interrelationships with microorganisms. The earthworms depend upon microorganisms as

38 their major source of nutrients, they promote microbial activity in decaying organic matter by
39 fragmenting it and inoculating it with microorganisms and they also disperse microorganisms widely
40 through soils [4]. Microflora get influenced by earthworms directly or indirectly through comminution,
41 burrowing, casting, grazing and dispersal which change the physico-chemical and biological status of
42 the soil or substrate which brings shifts in the density, diversity, structure and activity of microbial
43 and faunal communities [5]. Certain microbes may be enhanced, reduced or unaffected depending
44 on their ability to adapt to the earthworm drilosphere. It has been reported that the positive influence
45 of earthworms in increasing the population of two P-solubilizing bacterial inoculants during gut transit
46 and in vermicasts [6]. The present study reports such beneficial effects of earthworms on two P-
47 solubilizing diazotrophic bacterial isolates from the rhizosphere of finger millet which have been
48 shown to improve grain and straw yield in finger millet [7][8]. The vermicasts represent biopelletized
49 microbes in decomposed organic matter which act as inoculation loci when applied to the soils.

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51 **2. MATERIAL AND METHODS**

52 The studies were conducted in glass jars measuring 10 x 20 cm (W x L) with a substrate
53 holding capacity of 500 gm. The earthworm *Eudrilus eugeniae* was used. The composting material
54 was prepared by mixing equal quantities of green leafy material and cow dung. The material was
55 allowed for partial decomposition for three weeks. Later, the jars were filled with 500 gm of partially
56 decomposed material. To each jar, 0.5 gm of selected lignite based phosphate solubilizing
57 diazotrophic bacterial isolates *Bacillus* sp. (DT) and *A. chroococcum* (DT) were added separately and
58 mixed thoroughly. Eight medium sized earthworms were allowed in to each jar for worm working.
59 Moisture was maintained at 50-60 per cent throughout the period of study. The inoculant population
60 in the gut of earthworm and in vermicasts was estimated by dilution plate method using Pikovskaya's
61 and Jenson's agar medium. Two sets of jars were kept for each bacterial isolate, ten replicates each
62 to study the population in the gut and collecting vermicasts on 2nd, 20th, 40th and 60th day. The
63 earthworms in the first set of jars were used to study the phosphate solubilizing diazotrophic bacterial
64 population in the gut. The second set was used to study the inoculant population in the vermicasts.

65 To study the gut flora, the earthworms from each jar were separately dissected and the fore gut, mid
66 gut and the hind gut regions were removed, weighed and macerated in separate sterile test tubes and
67 appropriate dilutions were prepared in distilled and sterilized water. From appropriate dilutions, 0.1 ml
68 of suspension was plated and spread on Pikovskaya's and Jenson's agar media separately to
69 estimate the population. The vermicasts were collected on 2nd, 20th, 40th and 60th day from each of
70 the jars maintained for the purpose. One gram of vermicasts were placed in 100 ml distilled and
71 sterilized water and stirred on a magnetic stirrer for 30 minutes. Appropriate dilutions (0.1 ml) were
72 plated separately on Pikovskaya's and Jenson's agar media and spread with a sterile spreader. The
73 plates were incubated at 28-30 °C for three days in an incubator and the colony forming units were
74 counted. The population was estimated per gram of the sample. The population increase in the gut
75 regions were estimated against their initial population in the feeding material while those in the
76 vermicasts were compared against the population in the two day old vermicasts.

77 **3. RESULTS AND DISCUSSION**

78 The results on the population of bacterial isolates in the gut and vermicasts are presented in
 79 the following Table. The bacterial inoculants were stimulated in the gut and vermicasts recording
 80 significant increase in their population. Gradual increase in their population was recorded in the gut
 81 with highest population in hindgut region. The population of *Bacillus* sp. (DT) and *Azotobacter*
 82 *chroococcum* (DT) increased by 22.14, 42.14 and 97.92 percent in the fore gut, mid gut and hind gut
 83 regions respectively and the corresponding figures for *A. chroococcum* (DT) were 21.21, 41.19 and
 84 110.95 percent as compared to their initial population in the feeding material (4.20×10^3 cfu's/gm)
 85 (Fig.1).

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

Isolate	Population (cfu's/g) $\times 10^3$			Age of vermicasts (in days) and population (cfu's/g) $\times 10^4$			
	Fore gut	Mid gut	Hind gut	2	20	40	60
<i>Bacillus</i> sp. (DT)	5.13	5.97	8.30	10.37 (3.5)	30.79 (6.10)	36.00 (5.17)	16.30 (2.10)
<i>Azotobacter chroococcum</i> (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

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89 *Initial inoculant population: 4.20×10^3 cfu's/gm of composting material*

90 *Figures in parenthesis are the population in uninoculated control*

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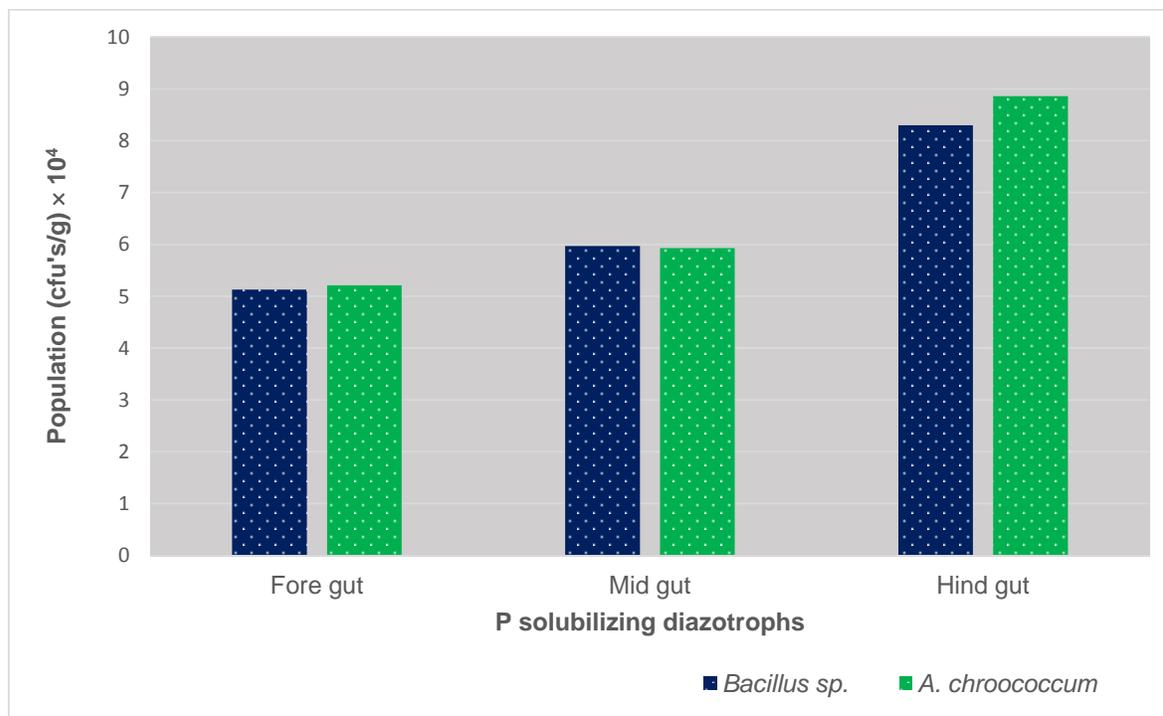
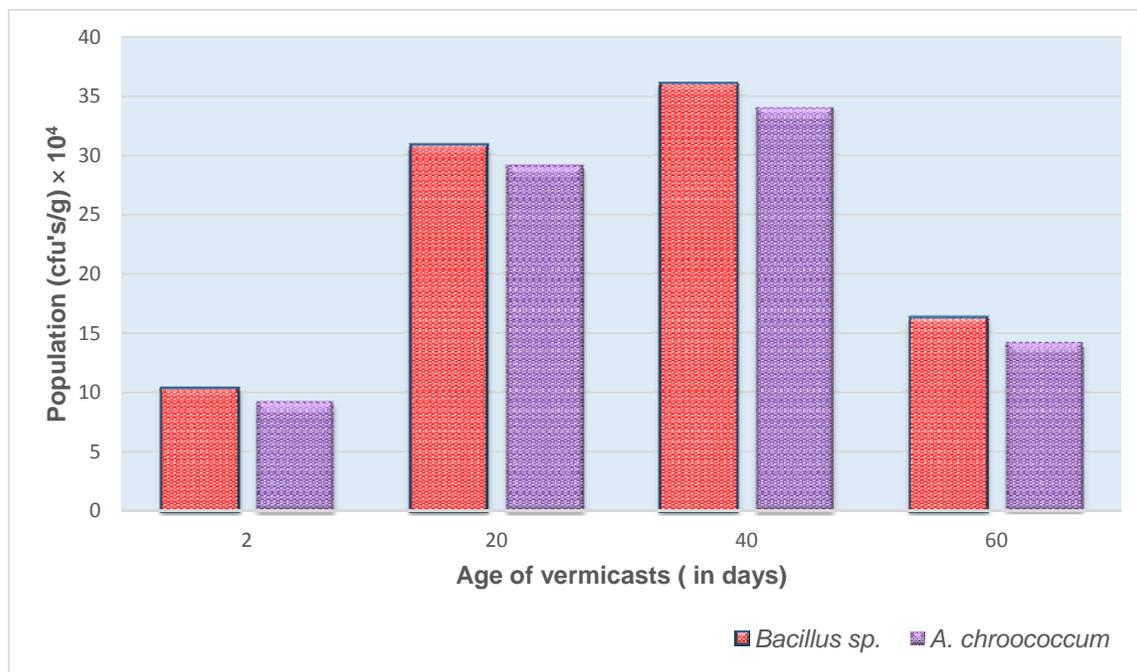


Fig.1. Population of P solubilizing Diazotrophs in the gut region of *Eudrilus eugeniae*

Enormous amount of increase in the isolate population was recorded in the vermicasts up to 40th day which later declined significantly in 60 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 per cent as compared to its population on 40th day. Similarly, *Azotobacter chroococcum* (DT) increased by 217.34 and 270.77 per cent on 40th day but declined by 58.24 per cent on 60th day as compared to their population on 40th day (Fig.2).



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106 **Fig. 2. Population of P solubilizing isolates in relation to age of vermicastes**

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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 vermicastes [14]. Vermicastes 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 not all the bacterial isolates survive the passage through earthworm gut [6].

REFERENCES

- [1] Doan TT, Ngo TT, Rumpel C, Nguyen BV, Jouquet P. Interactions between compost, vermicompost and earthworms influence plant growth and yield: A one year greenhouse experiment. *Sci. Hortic. Amsterdam*.2013; 160:148-154.
- [2] Tunira Bhadauria, Krishnan Gopal Saxena. Role of earthworms in soil fertility maintenance through the production of biogenic structures. *Applied and Environmental Soil Science*. 2010; 1-7.
- [3] Clive AE, Fletcher KE. Interactions between earthworms and microorganisms in organic matter breakdown. *Agriculture, Ecosystems and Environment*. 1988; 24(1-3): 235-247.
- [4] Edwards CA and Bohlen PJ. *Biology and Ecology of Earthworms*. Chapman & Hall Publishers, Madras, 1996:185-195.
- [5] George G Brown 1995. How do earthworms affect microfloral and faunal community diversity?. *Plant and Soil*; 1995; 170 (1): 209-231.
- [6] Sukumar J, Thimma Reddy H, Padma SD, Bongale UD. Earthworm for biopelletisation of biofertilizer inoculants. In: Bongale UD, Raghuraman R, Mallikarjunappa.RS (Eds.)/ *Bioorganics in sericulture and related technologies*. Karnataka State Sericulture Research and Development Institute publication, Bangalore. 2005; 42-45.
- [7] Chandana M, Venkataramana GV. Influence of phosphate solubilizing *Azotobacter chroococcum* on growth and yield of finger Millet (*Eleusine coracana* (L.) Gaertn.) under graded levels of N & P Fertilizers. *Trends in Biosciences*. 2018; 11 (36): 3930-3933.
- [8] Chandana M, Venkataramana GV. Response of finger millet (*Eleusine coracana* (L.) Gaertn.) to phosphate solubilizing diazotrophic *Bacillus* Sp. under graded levels of N & P Fertilizers. *Trends in Biosciences*. 2018; 11 (47): 4364-4367.
- [9] Stockli A. Studien uber den Einfluss der Regenwurmer auf die Beschaffenheit des Bodens. *Landw. Jb. Schweiz*. 1928; 42: 41-53. Cited from Edwards, C.A and Bohlen, P.J. 1996. *Biology and ecology of earthworms*. Chapman and Hall publishers, Madras, 181-195.
- [10] Parle JN. Microorganisms in the intestines of earthworms. *J. Gen. Microbiol.*1963; 31: 1-13.
- [11] Parle JN. Activities of microorganisms in soil and influence of these on soil fauna, Ph.D thesis, University of London. 1969. Cited from Edwards CA and Bohlen PJ 1996. *Biology and ecology of earthworms*. Chapman and Hall publishers, Madras; 1996: 181-195.
- [12] Teotia SP, Duley FL, McCalla TV. Effect of stubble mulching on number and activity of Earthworms. *Neb. Agric. Exp. Stn. Res. Bull.* 1950; 20.
- [13] Kollmannsperger F. Lumbricidae of humid and arid regions and their effect on soil fertility. 6th Congr. *Int. Sci. Sol Rapp. C.*, 1956; 293-297.
- [14] Barios I, Lavelle P. Changes in respiration rate and some physicochemical properties of a tropical soil during transit through *Pontoscolex corethrurus* (glossoscolecidae oligochaeta). *Soil Biology & Biochemistry*. 1986; 18 (5): 539-541.

[15] Scheu SS. Mucus excretion and carbon turnover of endogenic earthworms. *Biol. Fert. Soil.* 1991; 12: 217-220. Cited from Edwards CA, Bohlen PJ. *Biology and ecology of earthworms.* Chapman and Hall publishers, Madras, 1996; 181-195.

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