Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(4):264-267

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Enumeration and detection of phosphate solubilizing bacteria from the gut of earthworm varieties

N. Uma Maheswari* and S. Sudha

P.G and Research Department of Microbiology and a Division of Biotechnology, S. T. E. T. Women's College, Mannargudi, Thiruvarur, Tamilnadu (India)

ABSTRACT

Phosphate solubilizing bacteria (PSB) were isolated and enumerated from the gut of earthworm varieties, epigeic (Eisenia foetida) , Endogeic (Allolobophora chlorotica), anecic (Apporectodea longa). Total bacterial count and PSB were isolated and identified.Among 4 PSB isolates from three varieties of earthworm guts, highest number of PSB were isolated and enumerated from (Eisenia foetida). The dominant genera identified by cultural and morphological and physiological characteristics were Bacillus subtilis (23.5+2 mm) followed by Pseudomonas aeruginosa (12.2+01 mm) Enterobacter aerogens (18.3+04 mm) and P. fluorescens (11.2+0.01). Besides from this other genera like Klebsiella pneumoniae and E.coli were also identified, but in low frequency . Revealed that their higher number of PSB associate with earthworm gut . The present work follows the changes in the phosphate solubilizing in ingested material as it passes through the worm and attempts to evaluate benefits the worm may derive there form.

Keywords: Phosphate solubilizing bacteria (PSB), Epigeic, endogeic, anecic, earthworm gut.

INTRODUCTION

Earthworms are classified into epigeic, anecic and endogeic species based on definite ecological and trophic functions **[1]**. Epigeic earthworms are smaller in size, with uniformly pigmented body, short lifecycle, high reproduction rate and regeneration. They dwell in superficial soil surface within litters, feeds on the surface litter and mineralize them. They are photophagous and rarely ingest soil. They contain an active gizzard which aids in rapid conversion of organic matter into vermicompost. In addition epigeic earthworms are efficient bio-degraders and nutrient releasers, tolerant to disturbances, aids in liter communication and early decomposition and hence can be efficiently used for vermicomposting. Epigeic earth worms includes *Eisenia foetida, Lumbricus rubellus, L. castaneus, L . festivus, Eiseniella tetraedra, Bimastus minusculus, B .eiseni, Dendrodrilus rubidus, Dendrobaena veneta, D . octaedra*. Endogeics earthworms are samll to large sized worms, with weakly pigmented body, life cycle medium duration, moderately tolerant to disturbance, forms extensive horizontal burrows and they are geophagous feeding on particulate organic matter and soil. They bring about pronounced changes in soil physical structure and can efficiently utilize energy from poor soils, hence can be used for soil improvments. Endogeics include *Aporrectodea caliginosa, A . trapezoides, A. rosea, Millsonia anomala, Octolasion cyaneum, O.lacteum, Pontoscolex corethrurus, Allolobophora chlorotica* and *Aminthas* sp. They are further classified into polyhumic endogeic which are small sized, rich soil feeding earthworms, dwelling in top soil (A1); mesohumic endogeic whilch are medium sized worms, dwelling in A and B horizon, feeding on bulk (A1) soil; and oligohumic endogeic

which are very large worms, dwelling in B and C horizons, feeding on poor, deep soil Anecics are larger, dorsally pigmented worms, with low reproductive rate, sensitive to disturbance, nocturnal phytogeophagous, bury the surface litter, forms middens and extensive deep, permanent vertical burrow, and live in them. Formation of vertical burrows affects air,water relationship and movement front deep layers to surface helps in efficient mixing of nutrients. *Lumbricus terrestris, L . polyphemus* and *Apporectodea longa* are examples of aneceies earthworms [**2].**

Literature provides sufficient evidence of the presence of heterotrophs like bacteria, fungi, actinomycetes, etc,. in the gut of soil earthworm **[3].** Earthworm casts contained higher microbial populations and enzyme activities than the soil. Earthworm have higher amounts of N,P,K and organic C than Soil (P=0.05). Selective feeding by earthworms on organically rich substrates, which breakdown during passage through the gut, is likely to be responsible for the higher microbial populations and greater enzyme activity in the casts **[4].**The present work was planned to isolate PSB from the gut of earthworm varieties.

Earthworm castings having Phosphate- solubilizing activity but there was no detail of their antimicrobial activity, Phosphate - solubilizing microbes play fundamental roles in bio-geochemical phosphorus cycling in natural and agricultural ecosystems. Phosphate - solubilizing microbes can transform the insoluable phosphorus to soluble forms HPO_4^{2-} and $H_2PO_4^-$ by acidification, chelation, exchange reactions, and polymeric substances formation **[5]** .

EXPERIMENTAL SECTION

Collection of Earthworms

Earthworm varieties epigeic *(Eisenia foetida),* endogeic *(Allolobophora chlorotica),* anecic *(Apporectodea longa),* were collected from various garden Pattukkottai Taluk, Thanjavur District, Tamilnadu. Earthworms were washed with sterile tap water and placed on a petriplate moistened with filter paper and subjected to starvation for 24 hrs. After starvation the earthworms were then disinfected with 70% ethanol, and gut content was dissected out, homogenized (for 5 minutes in a vortex mixer) in sterile 0.85% NaCl Solution for isolation **[6].**

Isolation of bacteria from Earthworm gut

The gut contents [3-4 cm of gut ranging from 20-130 segments in *(Eisenia foetida), 5-5.5* am of gut ranging from 18-185 segments in *(Allolobophora chlorotica),* 2.5 – 3 cm of gut ranging from 17 – 100 segments in (*Apporectodea longa)*], of each species earthworms **[7]** *g*ut homogenate was serially diluted were placed on National Botanical Research Institute's phosphate growth medium (NBRIP) and incubated at 37^o C for 5-7 days for the isolation of bacteria. NBRIP medium containing tricalcium phosphate (TCP) as sole phosphorus source for selectively screening the bacteria which have the ability to release inorganic phosphate from tricalcium phosphate. After 5 days of incubation at 37^oC, PSB developed clear zones around colonies. Colonies with clear zones were further purified by replating on agar medium supplemented with TCP **[8].**

Identification of Bacteria

The morphological and biochemical tests were done by the methods described in Experiments in Microbiology, Plant Pathology and Biotechnology [**9].**

Gram staining

A thin smear of bacterial isolates were separately made on a clean glass slide and heat fixed. Then the smear was stained by crystal violet for one minutes and then washed with water followed by flooded with gram's iodine. After one minutes the slide was washed again it tap water and decolorized with alcohol. After decolorization the smear was counter stained with saffranin for one minutes. The slide was washed and air dried, finally it was observed under microscope **[10].**

Motility Test

A ring of petroleum jelly was applied around the concavity of the depression slide. A loopful of isolates was placed separately in the centre of a clean cover slip by using sterile technique. The depression slide was placed with the concave surface facing down over the cover slip and pressed gently to form a seal between the slide and cover slip. Then the slide was quickly turned the right side up. So that, the drop continuous to adhere the inner surface of the cover slip. Then the slide was observed through the oil immersion microscope [**11].**

Biochemical characteristics
The biochemical tests were conducted by the following methods as described by identify the bacteria [**12].**

Enumeration of total microbial population
The total microbial population was enumerated on NBRIP medium. The colony forming units (CFU) developing on the media were estimated and expressed as CFU x $^{10-6}$ g-1 respectively. The phosphate solubilizing bacteria and other bacteria were isolated and enumerated from the gut of *Eisenia foetida, Allolobophora chlorotica, Apporectodea longa* by using colony counter [**13] .**

Determination of P solubillization activity
Phosphorus solubilizing activities of each isolatewas assayed by spotting 10 l of cultures on the top of NBRIP media plates. The plates were incubated at 30C for one week and measured **[14].**

$$\text{Solubilization efficiency} = \frac{\text{Solubilization diameter}}{\text{growth diameter}} \times 100 \quad (1)$$

Table 1: Morphological characteristics of isolated phosphate solubilizing bacteria

S.NO	NAME OF THE ORGANISM	STAINING	SHAPE	MOTILITY
1.	*Pseudomonas aeruginosa*	Gram Negative	Rod	Motile
2.	*Bacillus subtilis*	Gram Positive	Rod	Motile
3.	*Pseudomonas fluorescens*	Gram Negative	Rod	Motile
4.	*Enterobacter aerogens*	Gram Negative	Rod	Non - Motile

Table 2 : Biochemical test for the identification of phosphate solubilizing bacteria

S.NO	Biochemical test	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Pseudomonas fluorescens*	*Enterobacter aerogens*
1.	Indole Production test	-	-	-	+
2.	Methyl red test	-	-	-	+
3.	Voges Proskauer Test	-	+	+	-
4.	Citrate Utilization	+	+	+	+
5.	Catalase test	+	+	-	_
6.	Urease test	+	_	-	+

Positive (+), Negative (-)

Table 3 : Morphology and biochemical characterization of isolated other bacteria

S.NO	*Morphology And Biochemical Characteristics*	*Escherichia coli*	*Klebsiella pneumoniae*
1.	Gram's staining	Gram Positive	Gram Negative
2.	Motility	Motile	Non motile
3.	Shape	Rod	Rod
4.	Indole production test	+	-
5.	Methyl red test	+	-
6.	Voges Proskauer test	-	-
7.	Citrate utilization test	-	+
8.	Catalase test	+	+
9.	Urease test	-	-

Positive (+), Negative (-)

Table 4 :Enumeration of total microbial population

Earthworm	Phosphate Solubilizing Bacteria (CFU X 10^{-6} g^{-1})	Other Bacteria (CFU X 10^{-6} g^{-1})	Toatl Microbial Population (CFU X 10^{-6} g^{-1})
Epigeic *(Eisenia foetida)*	38	15	53
Endogeic *(Allolobophora chlorotica)*	34	12	46
Anecic *(Apporectodea longa)*	23	7	30

Table 5 :Phosphate solubilization

S.No	Phosphate SolubilizationMicrooganisms	Phosphate Solubilization (mm)
1.	*Bacillus subtilis*	23.5±02
2.	*Pseudomonas aeruginosa*	13.2±01
3.	*Pseudomonas fluorescens*	11.2±0. 01
4.	*Enterobacter aerogens*	18.3±04

Values are expressed as mean± standard deviation

RESULTS AND DISCUSSION

In our study, the Phosphate solubilizing organisms were isolated and enumerated the gut of earthworms (*Eisenia foetida,) (Allolobophora chlorotica*), *(Apporectodea longa).* The Phosphate solubilizing organisms were isolated and identified by the Morphological and biochemical test. Phosphate solubilizing bacteria grow on the NBRIP medium. Enumerate the total microbial colonies developing on the media were counting by colony counter and expressed as CFUx10^6g-1. In our present study, four isolated Phosphate solubilizing bacteria strains were tested for phosphate solubilization activity in NBRIP media Plates, a clear halo zone indicating P Solubilizing activity. The zone is formed due to the phosphate solubilizing organisms which cleave[the Phosph*a*te molecules present in the media. In overall phosphate solubilization activity, total microbial population was maximum in *Eisenia foetida* followed by *Allolobophora chlorotica* and *Apporectodea longa* [**15].** Thus the role of microbes- earthworms throws light on the flux of nutrients, particularly trace elements, between microbes- earthworms- plants.

REFERENCES

[1] G.G. Brown, *Plant soil*, **170** : 209-231 (**1995).**
[2] Y. Kooch and H. Jalivand, *Pak J Biol. Sci*., **11** : 819-825 **(2008)**.
[3] G.R. Karten and Drake ,*Appl. Environ. Microbiol*., **61** : 1039-1044 **(1995).**
[4] S. C Tiwari and B.K Tiwari, *Biofer.. soil*, **8 :** 178-182 **(1989).**
[5] P. Delvasto and M.L. Garcia,. *Soil Bio. Bioche..*, **38:** 2645-2654 **(2006).**
[6] K. Toyoto and Kimura, *Biol. Fertil.Soil***, 18** : 32-36 (**1994).**
[7] K. Parthasarathi and L.S . Renganathan., *J . Environ. Biol*, **28 (1) :** 87-92 **(2007).**
[8] C. S. Nautiyal, *FEMS Microbiol. Lett*., **170** : 193 -199 **(1999).**
[9] K.R. Aneja, *Procedure for identification* , New age International publishers, New Delhi, India, **(2004).**
[10] Han's Christian Gram, *J. of Bact.*, **15**6**(2) :** 846-858 **(1884).**
[11] R.W. Bailey and B.G. Scoot , *Procedure for Motility Test* , St. Louis: The C.V. Mos by company. New York, 48-50 (**1966).**
[12] Norris and Ribbons, *Biochemical tests.* Methods in Microbiology. Vol**. 6 (1972).**
[13] C. Nguyen and W. Yan,and F. Le Tacon , *Plant soil*. **143** : 193- 199 **(1992).**
[14] K. Parthasarathi and L.S . Renganathan., *J . Environ. Biol*, **28 (1) :** 87-92 **(2007).**
[15] N. Pradhan and L. B. Sukia, *Afr. J. Biotechnol*., **5** : 850-854 **(2005).**