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Excellent N-fixing and P-solubilizing traits in earthworm gut-isolated bacteria: A vermicompost based assessment with vegetable market waste and rice straw feed mixtures

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HIGHLIGHTS

- *E. fetida* superseded *P. excavatus* in stabilizing vegetable waste and rice straw.
- 5 N-fixing and 3 P solubilizing bacteria were identified from earthworm guts.
- N-fixing ability of *Kluyvera* was a novel finding.
- Both N-fixing and P-solubilizing ability of *Serratia* was another rare observation.

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ABSTRACT

Vermicomposting is a dependable waste recycling technology which greatly augments N and P levels mainly through microbial action. This paper aims to identify efficient N-fixing (NFB) and P-solubilizing (PSB) bacteria from earthworm intestines. Various combinations of vegetable market waste, rice straw, and cowdung were fed to two earthworm species (*Eisenia fetida* and *Perionyx excavatus*). Total organic C decreased, pH shifted towards neutrality, and NPK availability, and microbial (NFB, PSB, and total bacteria) population increased remarkably during vermicomposting with *E. fetida*. Therefore, 45 NFB and 34 PSB strains isolated from *Eisenia* gut were initially screened, their inter-dominance assessed, and 8 prolific strains were identified through 16SrRNA sequencing. Interestingly, two novel N-fixing strains of *Kluyvera ascorbata* emerged as an efficient biofertilizer candidate. Moreover, both N-fixing and P-solubilizing strains of *Serratia* and *Bacillus* were isolated from earthworm gut. All the isolated strains significantly improved soil health and facilitated crop growth as compared to commercial biofertilizers.

1. Introduction

Remarkable acceleration in the rate of solid waste generation has been observed in all parts of the world in last few years, while the growth is more alarming in developing nations than the developed ones (Hoornweg and Bhada-Tata, 2012; Rockson et al., 2013). According to a recent estimate, biodegradable fractions range from 59% to 64% of the total solid waste generated in low to medium income nations (Hoornweg and Bhada-Tata, 2012). Vegetable (VW) and agricultural wastes like rice straw (RS) constitute a large portion of the total biodegradable fraction of solid wastes (Hu et al., 2016). Owing to high biodegradability and moisture content, VW and RS are often utilized for sustainable production of several useful chemicals and good quality fuels (Gulhane et al., 2016; Hu et al., 2016). In this context, vermicomposting can be a highly sustainable option to utilize these wastes because of the simplicity and economy of the technology for stabilization of complex solid wastes (Deka et al., 2011; Das et al., 2015). However, information on vermicomposting of VW and RS is rather scanty.

The biochemical decomposition of waste materials is primarily attributed to the microbial activity during vermicomposting; wherein the earthworms contribute to fragmentation and conditioning of the substrate which stimulate the microbial population thereby increasing the surface area available for microbial activity

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(Sen and Chandra, 2009). Interestingly, the community structure and the abundance of the microbial population could be greatly altered in vermireactors depending on the earthworm species and nature of the feedstock (Huang et al., 2013; Goswami et al., 2014). The participation of microorganisms within the digestive tract of the earthworms is of great importance as they play the key role in degrading solid wastes and stabilizing nutrient availability in the processed materials. Recently, Singh et al. (2015) presented a comprehensive survey on abundance and diversity of gut bacterial flora in *Eisenia fetida* and *Perionyx excavatus* applying *16S rRNA* based clonal technique. However, little is known about the earthworm gut microorganisms that are efficient N fixer and/or *P solubilizer*. Moreover, how microbial diversity in the earthworm gut varies with feedstock composition among earthworm species is yet to be studied systematically.

Under these perspectives, the present study was designed to isolate productive N fixing (NFB) and P solubilizing (PSB) bacteria from the intestinal microbiota of *Eisenia fetida* and/or *Perionyx excavates*, reared in vermibeds composed of various combinations of VW, RS, and cowdung (CD), on the basis of their efficiency in stabilizing the feed mixtures. Such performance was assessed in regard to nutrient (N, P, and K) availability, microbial diversity, and enzyme activity in the vermibeds. Total 45 N-fixing (NFB) and 34 P-solubilizing (PSB) bacterial strains were screened and based on their dominance, 8 strains were characterized through Gram staining and 16 S rRNA gene sequencing. In addition, the potential of the isolated bacterial cultures as independent nutrient augmentation reagent (i.e. biofertilizers) for agricultural crops was verified. The major objective of this work was to identify efficient N-fixing and P-solubilizing bacteria from earthworm intestines which can be used as biofertilizers in agriculture.

2. Materials and method

2.1. Experimental set up and sample collection

Fresh vegetable wastes (VW) [basic properties: bulk density (BD) = 0.72 g cc^{-1}; pH = 7.5; total organic C (TOC) = 1.14%; Mineralizable N = 67.33 mg kg^{-1}; available P = 22.12 mg kg^{-1}; available K = 157.6 mg kg^{-1}] were collected from waste disposal yard of vegetable market. While, rice straw (RS) [basic properties: BD = 0.76 g cc^{-1}; pH = 7.6; TOC = 2.14%; Mineralizable N = 40.00 mg kg^{-1}; available P = 28.07 mg kg^{-1}; available K = 104.6 mg kg^{-1}] and cowdung (CD) [basic properties: BD = 0.65 g cc^{-1}; pH = 7.3; TOC = 2.96%; Mineralizable N = 76.00 mg kg^{-1}; available P = 34.53 mg kg^{-1}; available K = 112.0 mg kg^{-1}] were collected from an agricultural farm situated nearby. Vermibeds were prepared with VW and RS chopped into pieces of about 1 cm × 1 cm size, mixed in various ratios, and 3 kg of each feed mixture was poured in perforated earthen vessels of dimension 0.45 m (width) × 0.45 m (length) × 0.30 m (width). Then, each feed stock was incubated independently with the selected earthworm species (*Eisenia fetida* and *Perionyx excavates*) @ 10 worm kg^{-1} and incubated for 2 months. Concurrently, identical sets of various treatment combinations were maintained for aerobic composting. Steady moisture content (50–60%) was maintained by intermittently sprinkling water and aeration was ensured by turning the feed mixtures daily. Samples were periodically drawn at 0, 30, and 60 days post incubation to assess the changes in physico-chemical properties in the aerobic compost and vermibeds. Watering was stopped at 55th day and on 60th day the earthworms were sieved and the composts was analyzed. The treatment combinations for the study were as below:

Vermicomposting		Aerobic composting
Eisenia fetida	*Perionyx excavatus*	
E1–CD only	P1–CD only	C1–CD only
E2–CD+RS (1:1)	P2–CD+RS (1:1)	C2–CD+RS (1:1)
E3–CD+VW (1:1)	P3–CD+VW (1:1)	C3–CD+VW (1:1)
E4–CD+RS+VW (1:2:2)	P4–CD+RS+VW (1:2:2)	C4–CD+RS+VW (1:2:2)

2.2. Analysis of physico-chemical changes during biocomposting and benefit ratio

The vermicompost and compost samples obtained from the vermibeds were analyzed for pH, bulk density (BD), total organic carbon (TOC), easily mineralizable nitrogen (Av. N), available phosphorus (Av. P), exchangeable potassium (Ex. K) following standard procedures (Page et al., 1982). Analytical grade chemicals (with more than 90% purity) were used, and all analysis was executed in accordance with the general quality control guideline.

The performance of the three biocomposting systems (*Eisenia* mediated vermicomposting, *Perionyx* mediated vermicomposting, and aerobic composting) were assessed to evaluate the potential benefit. The beneficial effect was computed for variables such as pH, Ex K, Av P and easily mineralizable N. The outcomes for each variable were assessed at the start (t = 0 day) and the end (t = 60 days) of each experiment by the following equation (Sahariah et al., 2015):

$$\text{Benefit ratio for N, P, K, and pH (BR)} = \frac{\text{Average concentration (60 d)} - \text{Average concentration (0 d)}}{\text{Average concentration (0 d)}} \quad (1)$$

$$\text{Benefit ratio for BD and TOC (BR)} = \frac{\text{Average concentration (0 d)} - \text{Average concentration (60 d)}}{\text{Average concentration (60 d)}} \quad (2)$$

2.3. Enumeration of microbial growth in compost and vermicompost samples

1 g of each air dried vermicompost and compost samples was suspended in 10 ml of de-ionized water and allowed to vortex for 15 min. Then, 0.1 ml of aliquots was serially diluted from 10^{-1} to 10^{-6} respectively and inoculated in petriplates following pour plate technique in nutrient agar (NA), Burk's, and Pikovskaya medium for enumeration of total, N-fixing (NFB), and P-solubilizing (PSB) bacterial population respectively (Parmer and Schmidt, 1964). Then, the petriplates consisting of NA were incubated for 24 h at 36 °C whereas the NFB and PSB plates were incubated for 48 h at 28 °C. Then, the colonies grown on the plates were counted with the help of a colony counter. The whole experiment was replicated thrice and the CFU (colony forming unit) ml^{-1} was calculated by the formula given below (Parmer and Schmidt, 1964):

$$\text{No. of bacteria ml}^{-1} = \frac{\text{No. of colonies} \times \text{dilution}}{\text{Amount plated}} \quad (3)$$