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Utilization Of *Bacillus* Sp. GGTD67 For Phosphate Enrichment In Vermicompost

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Natarajan Manivannan, Ramasamy Praveenkumar, Nooruddin Thajuddin, Thilagavathy Daniel and Muthukumaran Gunasekaran; Utilization Of *Bacillus* Sp. GGTD67 For Phosphate Enrichment In Vermicompost

ABSTRACT

Bacillus sp. is well known for their ability to mobilize phosphate from inorganic sources. The present study aimed at utilizing the *Bacillus* sp. GGTD67 to enrich the total phosphate content in vermicompost. Phosphate solubilizing *Bacillus* sp. GGTD67 was isolated from the rhizosphere of leguminous plants. Molecular characterization of the isolate was done through 16S rRNA gene amplification, BLAST search and multiple sequence alignment analysis. The culture was grown in mass and the cell count was done periodically. Pre-prepared vermicompost was mixed with suspension of *Bacillus* sp. at 1 x 10^8 cells/g. Treated vermicompost was mixed with soil, for pot culture studies. The composition of phosphate solubilizing bacteria with vermicompost showed higher productivity of the test plant *Vigna unguiculata* (L.) Walp. (cowpea).

Key words: *Bacillus* sp., Phosphate solubilization, 16S rRNA gene, *Vigna unguiculata* (L.) Walp.

Introduction

In recent days, organically raised products gained universal importance. It is well documented that the soil loses its natural fertility upon regular use of chemical fertilizers which gradually decreases crop yield. Under such conditions, use of vermicompost and biofertilizers are adopted as organic manure which has the potential to rejuvenate the soil with its lost nutrients and there by assures increased crop yield. Vermicomposting is a simple biotechnological process of composting, where earthworms are used to convert the organic wastes in to useful products [1]. It is a mesophilic process, utilizing microorganisms and earthworms that are active at 10 to 32°C. The process is faster than composting; where the waste material is ingested by the worms and which when passes through the gut gets transformed, whereby the resulting earthworm castings (worm manure) are rich in microbial activity and plant growth regulators. The earthworm *Eudrilus eugeniae* Kinberg is widely used for the decomposition of organic waste materials such as agricultural wastes, industrial wastes and household wastes for the production of vermicompost.

Phosphorous has been called the 'key of life' because it is directly involved in most of life processes. Next to nitrogen it is invariably classified as one of the macronutrients and is an important key element in frequency of use as fertilizer. Microbes which solubilize the bound phosphates and rock phosphates are called phosphate solubilizing microorganisms. Several soil bacteria particularly those belong to genera *Bacillus* possess the ability to bring insoluble phosphate into soluble forms. These organisms solublize the unavailable phosphates to the soil and thereby avail them to plants. Even though the growth stimulatory effects of either organic manure or phosphate solubilizing bacteria were routinely studied as a separate case [4,5], as per our knowledge no serious attempts, if so, very few were taken up to find the usefulness of introducing the phosphate solubilizing bacteria in vermicompost so as to stimulate the growth rate of plant.

In this background the present study aimed at analyzing the efficiency of *Bacillus* sp. (phosphate solubilizing bacteria) to enrich pre-prepared vermicompost and thereby stimulating the plant growth.

Materials and Methods

Organism:

Phosphate solubilizing bacteria *Bacillus* sp. GGTD67 used in this study was originally isolated

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from the rhizosphere of leguminous plants in Gandhigram, (Lat. 10°37' N / Lon. 78°01' E) Tamil Nadu, India. Molecular characterization of *Bacillus* sp. was carried out by 16S rRNA gene sequence analysis. The genomic DNA was isolated with a slight modification in the method described by Smoker and Barnum [2]. Followed by which amplification of 16S rRNA gene was carried out in a Long Gene thermal cycler (Model: MyGene 96G). Initial denaturation of template DNA was achieved at 92°C for 6 minutes. Further the cyclic denaturation was carried out at 92°C for 1min followed by cyclic annealing at 52°C for 50 sec, cyclic elongation at 72°C for 1 min for 35 cycles and a final elongation at 72°C for 7 minutes. The amplified product was sequenced and was compared with sequences in the GenBank database using BLAST. The CLC sequence viewer software, version 6.6.1., a bioinformatics tool was used for annotating multiple sequence alignments and thus to compare the retrieved sequences. The conserved domains among these sequences were also identified.

Mass Cultivation:

The phosphate solubilizing *Bacillus* sp. GGTD67 was cultivated in mass for pot culture studies. Their growth rate and cell viability were monitored at 48, 72 and 96 hours.

Pot Culture Studies:

Vigna unguiculata (L.) Walp. (cowpea) seeds used in this study were surface sterilized with 70 % sodium hypochlorite for 90 sec at room temperature**.** Vermicompost used in this study was prepared indigenously and the parameters of which were recorded (data not shown). *Bacillus* sp. GGTD67 culture suspension was mixed with vermicompost at the rate of 1 x 10^8 cells/g of vermicompost. The experimental design adopted in the present investigation is as described in table 1.

Table 1: Experimental design for pot culture studies with *Vigna unguiculata* (L.) Walp. (cow pea).

S. no.	Treatments	Notations
1	Seeds + plain soil (control)	T0
2	Seeds + soil mixed with vermicompost	T1
3	Seeds + soil mixed with vermicompost + *Bacillus* sp. GGTD67	T2

Statistical Analysis:

The statistical analyses were performed using SPSS Statistics 17.0 software (Statistical Program for Social Sciences 17.0). Mean and standard errors were calculated for the experimental analyses that were carried out in triplicates. Method of LSD and Duncan was employed to ascertain the values of the model parameters and ANOVA was used to establish their statistical significance at a confidence level of 99%.

Result and Discussion:

Bacillus sp. GGTD67 was grown in nutrient broth. This medium found to support their maximum growth as inferred from earlier studies. During their growth, it was observed that upon mixing the culture flasks, they readily form suspension in the medium. On Pikovskaya agar they formed clearing zones around the colony which indicates their ability to solublize inorganic phosphates. The time course of *Bacillus* sp. GGTD67 culture growth was shown in Fig. 1 constructed with cell count data obtained at 48, 72 and 98 hours over the course of cultivation. It was clearly inferred from the plot that the maximum cell density was obtained after 72 hours of cultivation. The cultivation yielded a maximum cell concentration of 5.92×10^9 cells/mL after 98 hours of cultivation with maximum cell viability of 90%. 16S rRNA gene product obtained from DNA sample extracted from *Bacillus* sp. GGTD67 displayed the expected size of about 600 bp (Fig. 2) which was further confirmed by sequencing. The BLAST analysis of the amplified sequence with other sequences in NCBI showed 97% similarity to the 16S rRNA gene sequences of some bacterial species. Multiple sequence alignments revealed the similarity between the studied sequences and the conserved domains among the studied sequences were represented in Fig. 3.

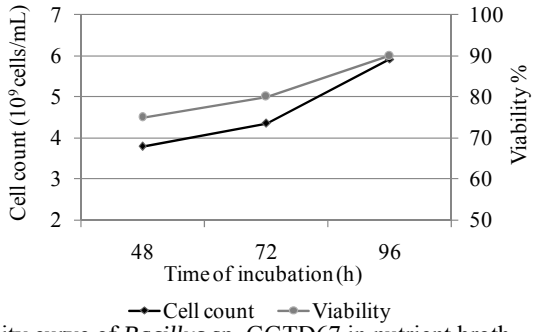

Fig. 1: Time course and viability curve of *Bacillus* sp. GGTD67 in nutrient broth.

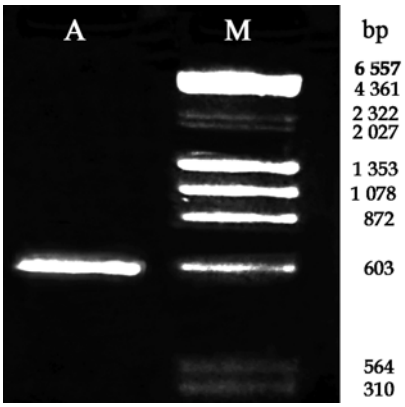

Fig. 2: Electrophoresis separation (1% (wt/vol) agarose gel) of the PCR products (about 600 bp) amplified from DNA extracted from *Bacillus* sp. GGTD67 targeting 16S rRNA gene. Lane A - *Bacillus* sp. GGTD67, lane M - 1kb ladder.

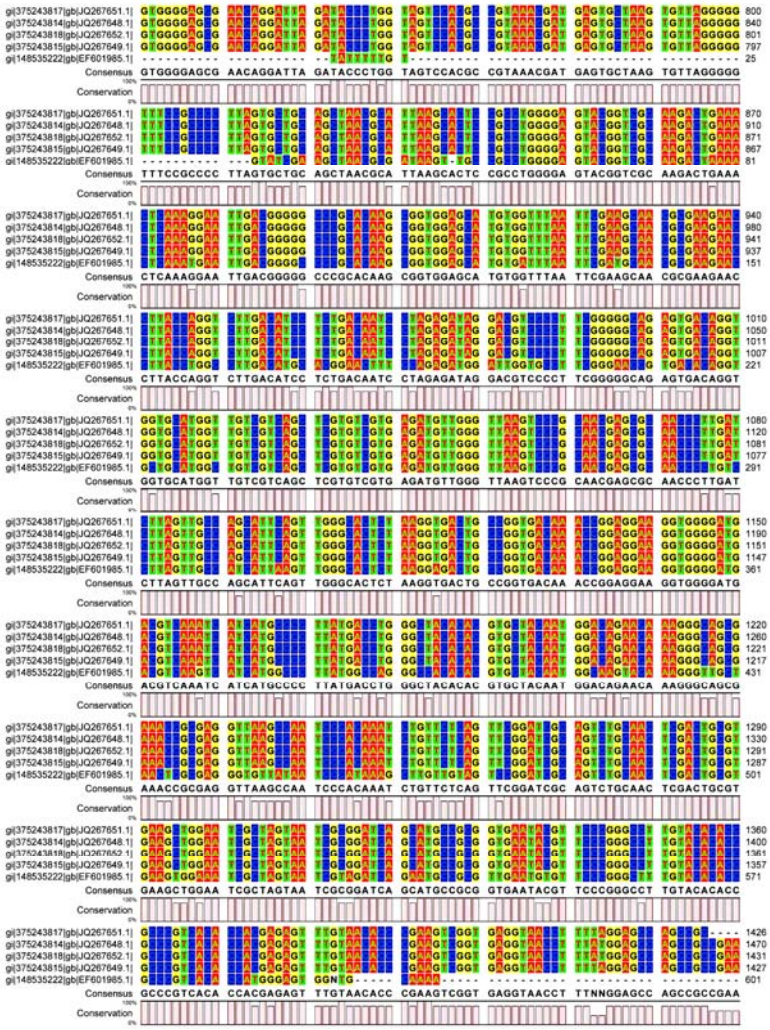

Fig. 3: The tabular format of a multiple alignment of 16S rRNA genes of *Bacillus* sp. GGTD67 and other related species from the NCBI database using the CLC sequence viewer software, version 6.6.1. First four being the reference sequences from NCBI database and the last sequence is of *Bacillus* sp. GGTD67.

As inferred from table 2, results of pot culture experiments revealed the superiority of treatment T2. The maximum yield performance of cow pea was observed through *Bacillus* sp. GGTD67 enriched vermicompost supplements. This is because of the more phosphorus content available in the soil formulation. Vermicastings either alone or in combination with microbial inoculants were able to initiate rooting and development of roots better than control. This is similar to the previous report which suggests that the effects of organic fertilizers alone may not stimulate the growth parameters of a plant [4], instead upon supplementation along with phosphate solubilizing bacteria such as *Bacillus* sp. GGTD67 as in our case, significant increase ($P < 0.01$) in both morphometric and yield parameters were observed (Fig. 4). This is most likely due to the ability of phosphate solubilizing *Bacillus* to bring insoluble phosphate into soluble forms by secreting organic acids such as formic, acetic, propionic, etc. These acids lower the pH and bring about the dissolution of bound forms of phosphate. Some of the hydroxyl acids may chealate with calcium and iron resulting in effective solubilization and utilization of phosphates [3].

Table 2: Morphometric and yield parameters of cowpea (*Vigna unguiculata* (L.) Walp.) under different organic formulations. T0 – Control, T1 – Vermicompost, T2 - Vermicompost with *Bacillus* sp. GGTD67. Values are means ± standard deviations of three replicates. Significant differences ($\alpha = 0.01$) were shown in superscript. Multiple superscripts indicate non-significant difference.

Treatments	Shoot length (cm)	Root length (cm)	No. of flowers	No. of pods	Pod wet weight (g)	Pod dry weight (g)
T0	13.7 ± 0.18^{a}	4.5 ± 0.09^{a}	14 ± 1^{a}	12 ± 1^{a}	1.4 ± 0.01^{a}	0.7 ± 0.01^{a}
T1	51.3 ± 0.25^{b}	13.8 ± 0.04^{b}	23 ± 2^{b}	20 ± 0^{b}	2.8 ± 0.04^{b}	0.95 ± 0.02^{ab}
T2	67.7 ± 0.32^{c}	19.4 ± 0.07^{c}	30 ± 1^{c}	28 ± 2^{c}	3.3 ± 0.03^{c}	1.24 ± 0.01^{b}

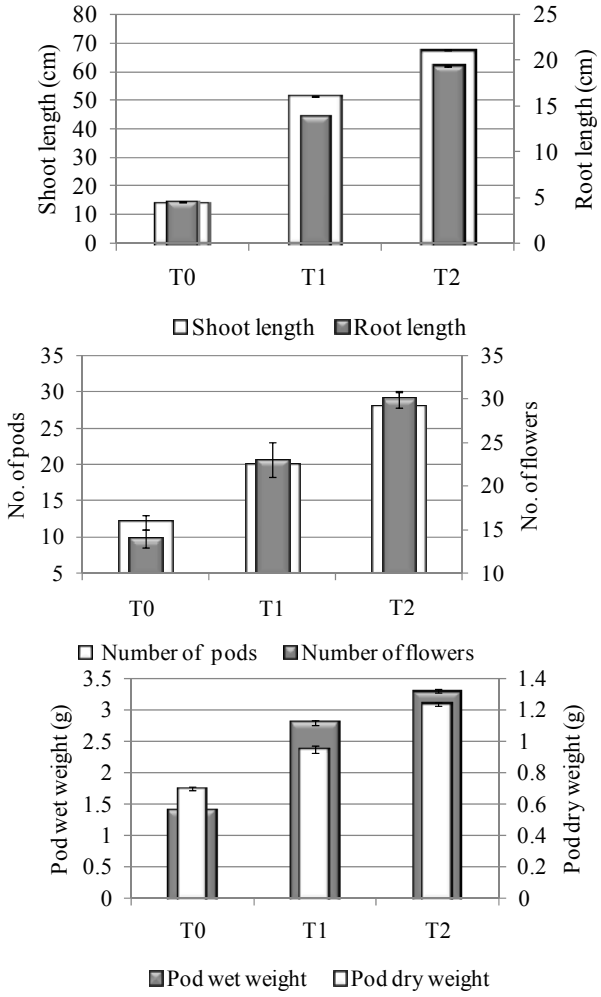

Fig. 4: Morphometric and yield parameters of cowpea (*Vigna unguiculata* (L.) Walp.) under different organic formulations. T0 – Control, T1 – Vermicompost, T2 - Vermicompost with *Bacillus* sp. Experiments were carried out in triplets.

Conclusion:

The results of this study indicate that the naturally isolated bacteria *Bacillus* sp. GGTD67 is a valuable candidate to be used as co-inoculants with vermicompost as a fertilizer, since it has high efficiency to mobilize inorganic phosphate to soluble forms. We speculate that the growth responses were due to the combined activity of growth promoting substances from vermicomposts and phosphate solubilizing *Bacillus* sp. GGTD67. We suggest that *Bacillus* sp. GGTD67 could be used in combination with vermicomposts. Obviously development of such a combinational organic fertilizer will help to cope with the potential need to rejuvenate the soil and thereby assuring increased crop yield.

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