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A study on biopotential of PGR producing bacterial species isolated from Vermicompost

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ABSTRACT

Vermicompost sample was produced by *Eudrilus eugeniae* in cowdung was collected from the Department of Biology, Gandhigram Rural Institute- Deemed University, Gandhigram. The sample was serially diluted and bacterial cultures were isolated and they were identified based on their characteristics as *Micrococcus* sp., *Alcaligens* sp., *Tricoccus* sp., *Azomonas* sp., and *Paracaccus* sp. These five bacterial isolates were subjected to screening for Indole acetic acid (IAA) production and all the bacterial colonies showed positive results for IAA production. Among the five isolates, *Alcaligens* sp. produced more amount of IAA (10µg/ml) when compared to all the other organisms. Hence *Alcaligens* sp was chosen for plant growth studies at 1 to 5 ml concentration. The isolate at 5 ml showed improved germination percentage of *Vigna unguiculata* seeds and it also showed improved shoot length (13.25 cm), root length (4.99 cm), number of leaves (5.0), chlorophyll content of leaves a-(0.0025 mg/g), b-(0.177 mg/g), total chlorophyll (1.351 mg/g), fresh weight (1.07 g) and dry weight (0.08 g) on the 7 day when compared to the control and the lower concentrations (1,2,3,4 and 5 ml).

Introduction

Soil is an abiotic factor, which inhabits varieties of microfauna. The microorganisms are interdependent and the nutrient cycling in the soil is highly influenced by the activity of these organisms to exploit the organic resources available in the litter and soil. The microorganisms mutually associate with macroorganisms like earthworms and vice versa (Moore, 1998; Levelle *et al*., 1995 and Daniel *et al*., 1999). The growth of plants depends mainly on the fertility status of the soil. In the tropical and sub-tropical regions, heavy leaching of nutrients from the layers of soil affects the fertility status of the soil (Lui-shuxin *et al.*, 1992). The major role of the earthworms in the soil is the decomposition of organic materials, developing soil structure and altering physico-chemical properties (Zhang and Schrader, 1993). During the process of vermicomposting, the earthworms decompose complex waste materials into simpler forms with the help of microorganisms. Vermicompost has been shown to have higher level of organic matter, organic carbon, total available NPK, other micro nutrients, microbial enzyme activities and plant growth regulators (Mulongoy, 1989; Karmegam and Daniel 2000-c; Manivannan and Daniel, 2008). Vermicompost have consistently improved seed germination, enhanced seedling growth and development, increased plant flowering, fruiting and productivity much more than could be possible from the mere conversion of mineral nutrients into plant-available forms (Atiyeh *et al*., 2002, Karmegam and Daniel, 2008-a). The dramatic increase in microbial activity in organic matter by earthworms could result in productions of significant quantities of plant growth regulators such as indole acetic acid (IAA), gibberellins and cytokinins (Edwards, 1998). Phytohormones are believed to assimilate in partitioning patterns of plants and affect the growth patterns of roots. Because of their regulatory role in plant growth and development, they are called as plant growth regulators (Tongmin.A *et al.*, 2006).There is also an evidence that the growth hormones produced by bacteria can increase the growth rates and improve the yield of host plants (Vijaya Ramesh, 2004).

The quantity of IAA produced and the sensitivity of the plant tissue also play an important role in several functions, such as root elongation and the formation of lateral adventitious roots. The present study was conducted to isolate the IAA producing bacterial species from vermicompost sample tested on the growth of *Vigna unguiculata* L walp.

Materials and Methods

Vermicompost prepared from cow dung medium using the earthworm, *Eudrilus eugenia* Kinberg was collected from the Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram, Tamilnadu.

The sample was serially diluted up to 10^{-6}, incubated at 37ºC for 24 hrs. Predominant colonies were identified based on colony morphology, staining and various biochemical tests using standard methods. The bacterial isolates *Micrococcus* sp., *Alcaligens* sp., *Tricoccus* sp., *Azomonas* sp., and *Paracaccus* sp. were screened for IAA production. After estimation the IAA produced by *Alcaligens* sp. was used to determine a 7 days plant growth promoting activity of cowpea, *Vigna unguiculata* (L.) Walp in paper cups in treatment 1 to 5 ml concentrations respectively. The germination percentages were calculated (Baki and Anderson, 2004 and Sheela *et al*., 2004). After 7 days shoot length, root length, number of leaves, chlorophyll content of leaves (a, b and total chlorophyll), fresh weight and dry weight of the whole plants were recorded.

Results

Isolation of bacterial colonies

The bacterial colonies from the vermicompost were $36x10^{6}$ CFU/g. The predominant bacterial strains isolated were

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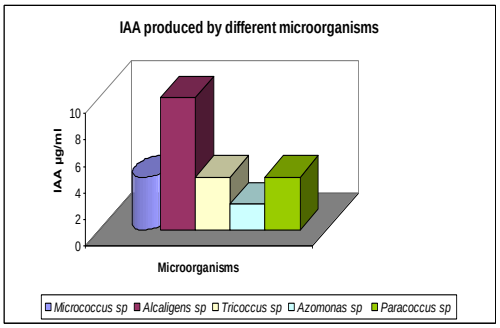
identified as: *Micrococcus* sp., *Alcaligens* sp., *Tricoccus* sp., *Azomonas* sp., and *Paracoccus* sp.

Screening of Bacterial Isolates for IAA Production

All the five bacterial isolates showed pink colour development during screening for IAA production and it indicates that all the bacterial isolates are capable of producing IAA. The spectrophotometric reading of the pink colouration produced by IAA showed 4 µg/ml in *Micrococcus* sp., 10 µg/ml in *Alcaligens* sp., 4 µg/ml in *Tricoccus* sp, 2µg/ml in *Azomonas* sp., and 4 µg/ml in *Paracoccus* sp. (Figure 1).

Figure 1.Screening of bacterial isolates



Evaluation of seed germination of cow pea, *Vigna unguiculata* (L.) Walp.

The results of the seed germination studies showed that *Alcaligens* sp., at 5ml concentrations capable of increasing the germination percentage (100) of *V. unguiculata.*

Pot culture study

The results of the seven days pot culture study showed that the bacterial strain, *Alcaligens* sp., at 5ml concentration (Treatment 5) enhanced the shoot length (13.25 cm), root length (4.99 cm), number of leaves (5.0), chlorophyll content of leaves i.e. a-(0.0025 mg/g), chlorophyll b-(0.0177 mg/g) and total chlorophyll content-(1.351 mg/g), fresh weight (1.07 g) and dry weight (0.08 g) of whole plant significantly when compared to the control: shoot length (4.31 cm), root length (1.5 cm), number of leaves (1.4), chlorophyll content of leaves a–(0.00047 mg/g), chlorophyll b–(0.0113 mg/g) and total chlorophyll –(0.730 mg/g), fresh weight (0.37 g) and dry weight (0.04 g) were lesser than Treatment 5 respectively (Table-1 and 2).

Discussion

Vermicomposting is the biotechnological process to stabilize the organic waste resources, involving the joint action of earthworms and associated micro-flora. The bacterial colonies were isolated and enumerated (CFU). Davis *et al.,* (1992) have enumerated the microorganisms from the composting. The predominant bacterial colonies were isolated and identified. The study shows that vermicompost carries the bacteriae which are capable of producing plant growth regulators (PGR) and such bacterial colonies grow as predominant colonies in vermicompost i.e., vermicompost function as a good medium to support that growth of PGR producing bacteriae. Krishnamoorthy and Vajrobhiah, (1986) also have absorbed the same results in their studies. However the quantity of IAA produced by these five different strains isolated from vermicompost varied from 2µm/ml as observed in the spectrophotometric reading and Ahamed *et al*., (2004) studies also indicates such variation. Based on the agricultural need vermicompost can be used as a medium to produce PGR microbes or used as such to improve growth in crop plants. Presence of *Alcaligens* sp., has promoted 100 percent germination in *V. unguiculata*, even at 1 ml concentration whereas it was only 80 percent in the control. Hence there is scope to enhance germination in legume like *V. unguiculata* in the presence of PGR producing microbe like *Alcaligens* sp. Sheela *et al*., (2004) and Madhainyan *et al*., (2004) also observed similar results in their experiments.

The seven day plant growth studies carried out in paper cups also showed a steady increase in the shoot length, root length, number of leaves, chlorophyll content and in fresh and dry weight with the increase in the concentration of PGR producing bacteriae culture applied to the growth medium. Edward and Burrows, (1998) and support for this observation. Even in the presence of lowest concentration of 1ml of *Alcaligens* sp. a significant increase in all the growth parameters was observed than in the control (*Alcaligens* free growth medium).

The study shows that there is scope to use the vermicompost borne PGR producing bacteriae as a supplement in the plant growth medium to enhance plant growth. More studies are needed in this field.

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Table 1 Plant growth study of cow pea *(Vigna unguiculata* (L.) Walp) using bacterial strain (*Alcaligens* sp) at different concentrations

Treatment	Germination percentage	Shoot length on 7th day (cm)	Root length on 7th day (cm)	No of leaves on 7th day
Control	80	04.31 ±1.355	1.5 ± 1.35	1.4 ± 1.78
T_1	100	09.37 ± 0.577	2.2 ± 1.52	4.4 ± 1.10
T_2	100	11.97 ± 0.69	2.4 ± 2.94	4.6 ± 1.17
T_3	100	12.72 ± 1.63	2.6 ± 2.88	4.7 ± 1.15
T_4	100	12.97 ± 0.88	3.2 ± 1.85	4.9 ± 0.87
T_5	100	13.25 ± 2.01	4.9 ± 2.50	5.0 ± 1.91

Table 2. Plant growth study of cow pea *(Vigna unguiculata* (L.) Walp) using bacterial strain (*Alcaligens* sp.) at different concentrations

Treatment	Chlorophyll content of leaves(mg/g)			Fresh weight on 7th day (g)	Dry weight on the 7th day (g)
	Chlorophyll -a	Chlorophyll -b	Total Chlorophyll		
Control	0.0004	0.0113	0.730	0.37 ± 0.36	0.04 ± 0.02
T_1	0.0014	0.0126	0.938	0.72 ± 0.21	0.06 ± 0.01
T_2	0.0014	0.0147	1.078	0.83 ± 0.32	0.06 ± 0.02
T_3	0.0015	0.0168	0.195	0.87 ± 0.24	0.06 ± 0.01
T_4	0.0017	0.0170	1.255	0.96 ± 0.38	0.07 ± 0.02
T_5	0.0025	0.0177	1.351	1.07 ± 0.20	0.08 ± 0.05

Control = Seeds inoculated with distilled water.
T1 = Seeds inoculated with 1ml of *Alcaligens* sp culture.
T2 = Seeds inoculated with 2ml of *Alcaligens* sp culture.
T3 = Seeds inoculated with 3ml of *Alcaligens* sp culture.
T4 = Seeds inoculated with 4ml of *Alcaligens* sp culture.
T5 = Seeds inoculated with 5ml of *Alcaligens* sp culture.