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ISOLATION AND IDENTIFICATION OF BACTERIAL STRAINS IN VERMICOMPOST AND VERMIWASH

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Abstract
Vermicompost is the excreta of the earthworms which are capable of improving the soil health and nutrient status. Earthworm activity is closely associated with microbial activity depending on the availability of rich biowastes in the medium. Standard microbial culture based methods were used to study the microbial diversity in Vermicompost and Vermiwash. Bacterial colonies were isolated from vermicompost and vermiwash samples using serial dilutions and plated onto general growth media. Gram staining and Biochemical analysis were conducted on the isolated bacterial strains from the samples. In the present study, the level of bacterial diversity and growth of large aerobic bacterial population in the compost supported the hypothesis that microorganisms in vermicompost and vermiwash may be able to maintain sustainable soil health for agricultural productivity.

Keywords: Microbial Activity, Isolation, Vermicompost, Vermiwash, Gram Staining, Bacterial Diversity.

Introduction

Earthworms are generally called as biological indicators of soil fertility. Earthworms play a vital role in maintaining soil quality and managing efficient nutrient cycling. Vermicompost is a method of making compost with the use of earthworms, which generally live in soil, eat biomass and excrete it in digested form. Earthworm manure is formed from the death tissues of plant and other animal biomasses like excreta of cow, goat, sheep etc and is naturally the source of macro ad micro nutrients .The soil rich with earthworms generally support healthy population of bacteria, fungi, actinomycetes, protozoans, insects, spiders, millipedes and a host of other organisms which are essential or sustaining a healthy soil [1] .

Vermicomposting differs from composting in several ways [2] It is a mesophilic process that utilizes microorganisms and earthworms that are active at 10°C to 32°C (not ambient temperature but temperature within the pile of moist organic material). The process is faster than composting; because the material passes through the earthworm gut, a significant but not fully understood transformation takes place, whereby the resulting earthworm castings (worm manure) are rich in microbial activity and plant growth regulators, and fortified with pest repellence attributes as well [3]. Vermicompost process creates a soil product with a rich bacterial community [4] which are capable of eliminating other bacteria or pathogens that are harmful to key crops [6] . Earthworms act as biological agents to consume the biodegradable wastes such as farm wastes, kitchen wastes, market wastes, bio-wastes of agro based industries and to deposit excreta by the process called vermicomposting [2].

Vermicomposting is considered as a simple biotechnological process of composting where certain species of earthworms are used to promote the process of conversion of waste product into a better product [1] .It is also considered as a mesophhilic process which utilizes microorganisms and earthworms that are active at 10^0 c to 32^0 c. Vermicompost are rich in microbial activity and plant growth regulators

[6,7] . Lab studies recently determined that standard compost is generated through the decomposition of solid animal or plant waste. This process creates a soil product with a rich bacterial community [5] which may be able to outcompete and eliminate other bacteria and pathogens that are harmful to key crops [6] . Vermiwash is a watery extract of vermicompost and has high impact on plant growth [7] Vermiwash is a organic liquid fertilizer, which has been reported to have excellent growth promoting effects besides serving as biopesticides [8] . Vermiwash contains several enzymes, plant growth hormones, vitamins along with micro and macro nutrients that increases the resistance power of crops against various diseases and also promotes the growth and productivity of crops [9]. In short, earthworms through a type of biological alchemy are capable of transforming garbage into "gold" [10, 11] . Vermicomposts have outstanding physico-chemical and biological properties with plant growth regulators and diverse microbial populations than the conventional thermophilic composts [12]. Vermicomposts are high in microbial diversity, exactly fungi, bacteria and actinomycetes [13].
The objective of this study was to gain a better understanding of bacterial communities inhabiting vermicompost made from cow manure, water hyacinth and grasses by isolating and identifying bacterial strains from the compost. Vermiwash and Vermicompost samples were diluted and bacterial strains were isolated, grown and analyzed by biochemical tests.

Materials and Methods

Collection of samples and Isolation of Bacterial Strains

The vermicompost and vermiwash samples were collected from the Vermicomposting Unit of D.K.D. College Dergaon, Golaghat district of Assam, India. The cowdung, water hyacinth, grasses were used as biowastes to fed the earthworm species of Eisenia fetida .

Using serial dilution, 1 ml of both samples were separately inoculated on Nutrient Broth Media followed by 24 hrs incubation at 37°C. After incubation 0.1ml of Nutrient Broth cultures of vermicompost and vermiwash samples were poured in the Nutrient Agar plates respectively. The bacterial colonies were picked up from each plate and sub cultured in Nutrient Agar plate till the pure culture in single colonies were obtained. The pure single colonies were identified and characterized for their colony and cell morphology, gram staining and biochemical tests. Identification of bacteria was based on the examination of slides of Gram stained microorganisms prepared from pure cultures grown on agar.

Biochemical Characterization

Indole Production Test

Indole Production test was used to determine the ability of the organism to produce Indole. Peptone Broth was prepared, sterilized and cooled. Inoculated the test organism to the sterile peptone broth and incubated the tubes at 37°C for 24 hrs. The culture was observed for the development of cherry red colored ring after the addition of Kovac's reagent indicating the positive result.

Catalase test

Catalase test was performed on all the purified isolates. With the help of a wooden stick, an isolated colony was transferred to a glass slide and a drop of 3% $H_2 O_2$ was added to observe3 the presence or absence of effervescence.

Methyl Red Test

Methyl Red test was employed to detect the ability of microorganisms to oxidize glucose with the production of high concentration of acid end products. The isolated organisms were inoculated into test tubes containing sterile MR-VP Broth and incubated the tubes for 24 hrs 37°C. After incubation, added

7-8 drops of Methyl Red Indicator to the culture tubes to observe the appearance of red color indicating the positive result.

Voges-Proskauer Test

This test is also known as the Acetoin Production Test. This test was used to differentiate the capacity of organisms to produce some non-acidic or neutral end product such as acetyl- methyl carbinol or 2,3,-butanediol. The isolated organisms were inoculated into sterile MR-VP Broth tubes and incubated for 24 hrs at 37°C. Development of deep rose color following the addition of Barritt's Reagent A and B indicated the positive result. The absence of deep rose color was a negative result.

Citrate Utilization Test

Some of the organisms were capable of utilizing Citrate as the sole carbon source and Mono ammonium phosphate at the sole source of nitrogen. As a result, the pH of the medium change, this was indicated by changes in the indicator present in the medium. Simmon's citrate medium was prepared, sterilized and kept in a slanting position and allowed the tubes to solidify. The test organisms was streaked on the slant and incubated at 37°C for 24 hrs. The change of color green to Prussian blue colored slant incubated the positive result.

Results and Discussion

After 24 hours of incubation, cream colored colonies were observed in the Nutrient Agar plates (Table:1 & Fig:1) indicating the presence of microbial population. Table:2 shows the morphological characteristics of isolated E. fetida vermicompost bacterial species. The bacterial strains were gram positive nature and rod in shape. Table:3 contained biochemical results of bacterial strains isolated from vermiwash and vermicompost of earthworm (Eisenia fetida) as shown in Fig: 2.

Vermicompost is an excellent soil additive that made up of digested compost. Worm castings are much higher in nutrients and microbial life. Therefore, it is considered as a higher value product. Vermicompost improves nutrient status of the soil. Eisenenia fetida earthworms were used to convert organic waste into dark brown nutrient-rich humus. It has been reported that vermicompost has an ability to fight soil-borne plant diseases and the humus also increases water permeability and water retention capacity, contributing to better plant health and more efficiently use in soil moisture. It is found that nitrogen concentrations are higher in vermicompost than in aerobic compost piles. Vermicomposting process improves soil aeration and thereby promotes the survival and dispersal of the useful bacterium within such systems.

"Vermiculture Movement" is going on in Assam with multiple objectives of agricultural and community waste management leading highly economical way of crop production. Vermicompoating is a ideal process for waste recycling and it will occur in a short period in Assam. Farmers of Assam urgently need a sustainable alternative, which is both economical and productive maintaining soil health and fertility. Vermicomposts have outstanding physico-chemical and biological properties with plant growth regulators and diverse microbial populations than the conventional thermophilic composts (Edwards et. al. 2004). Vermicomposts are high in microbial diversity, exactly fungi, bacteria and actinomycetes (Chaoui et. al. 2003).

Table: 1 Observation of Bacterial colony

Sl. No.	Observation
vermiwash sample	Cream coloured colonies were observed
Vermicompost sample	Cream coloured colonies were observed

Table: 2 Morphological Characteristics of Isolated Bacterial Strains

Sl. No.	Staining	Shape
Vermiwash sample	Gram negative	Rod
Vermicompost sample	Gram negative	Rod

Table: 3 Biochemical Test For The Isolated Bacterial Strains

Sl NO.	Biochemical Test	Vermiwash Sample	Vermicompost Sample
1	Indole test	+	+
2	Catalase test	+	+
3	Methyl test	+	+
4	Voges- Proskauer	-	-
5	Simon Citrate test	+	+

Fig-1: Bacterial colonies Isolated from Vermiwash & Vermicompost sample

Test Tube -1& 2 Indole test of VW & VC Sample: Test Tube-3&4 Citrate test of VW & VC Sample

Test Tube -5 & 6 Methyl Red test of VW & VC Sample

Test Tube -7 & 8 VP test of VW & VC Sample

Fig-2: Biochemical characterization of Isolated Bacterial strains

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