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Bacillus subtilus **isolation from insecticide (monocrotophos) treated** ***Eudrilus eugeniae*** **species vermicompost in laboratory culture**

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Abstract

The modern agriculture system 100% pollutes the soil vicinity problematic and sick, putting agricultural products at risk. To avoid this type soil contamination we are going to safe guard the soil organisms particularly earthworms. Because earthworms are play a lot of roles in soil vicinity. Mainly earthworms do composting work with combination to soil micro flora. Vermitechnology is a low cost and effective tool for environmentally sound municipal solid waste management. Earthworm species namely, *Eudrilus eugeniae* was used as a research tool in current research. Identification of bacteria was based on the examination of slides of Gram stained microorganisms prepared from pure cultures grown on agar agar. *Bacillus subtilus* strain isolated from a vermicompost site under the laboratory controlled system.

Keywords: Earthworm, Vermicompost, Bacteria, ***Bacillus subtilus***

Introduction

Soil, is the heart for farmers. The soil already enough quantity polluted by different activates. In the mean time, additionally for getting high crop yield and necessity to safe the crops from predators and pests, farmers are applying different kinds of pesticides, insecticides, herbicides, weedicides, fungicides, nematicides, bactericides, miticides, molluscicides, rodenticides, virucides, inorganic fertilizers, etc. Due to this activity our soil ecosystem problematic and sick, putting agricultural products at risk. To avoid this type soil pollution we are going to safe guard the soil organisms particularly earthworms. Because earthworms are play a lot of roles in soil vicinity. Mainly earthworms do composting work with combination to soil micro flora.

Association between earthworms and microorganisms seem to be complex. Earthworms are reported to have interaction with soil bacteria (Ismail 1995). Earthworms accelerate soil microbial activities (Binet *et al* 1998) and microbial biomass (Edwards and Bohlen 1996), by improving aeration through burrowing actions. Epigeics namely *Eudrilus eugeniae* have been used in vermiculture (Kale and Bano 1988).

Vermitechnology is a low cost and effective tool for environmentally sound solid waste management (Asha *et al* 2008). As the organic matter passes through the gut of the earthworm it is grounded into a fine powder after which the digestive enzymes and microorganisms act on them finally passes out as a casts, finally earthworm gut microbes converting them into mature product, the vermicomposts (Dominguez and Edwards 2004).

Vermicompost is a peat like material, exhibiting high porosity, aeration, drainage, water holding capacity, rich microbial activities and exhibit great plant growth (Subler *et al* 1998; Arancon *et al* 2004). Vermicomposts contain rich nutrient concentrations than composts. In addition, vermicomposts possess outstanding biological properties and have microbial load significantly larger compared to other conventional composts (Edwards 1998). The populations of microorganisms particularly bacteria was determined by using nutrient agar.

Bacillus subtilis is a gram positive, spore forming, fermentative, aerobic and rod shaped bacterium (Perez 2000). These are found mostly in soil, compost, fresh and marine water and play an important role in the decomposition of organic materials and produce secondary metabolites of commercial interest (Edwards 1998). *Bacillus subtilis* is a type of strain which is both taxonomically and metabolically diverse. These bacteria are capable of secreting antibiotics in great numbers to the exterior of the cell (Ara 2007). Therefore, present research was carried out to discuss the *Bacillus subtilis* bacterial biodiversity of vermicomposts.

Materials and Methods

Research Tool

Earthworm species namely, *Eudrilus eugeniae* was used as a research tool in current research. *E. eugeniae* was procured from Shakthi Vermicompost Farm in Vadipatti, Madurai district, Tamilnadu, India and kept on mother culture tank at Venture Institute, Madurai that was moist with de-ionized water with periodical interval. The cow dung was added in the soil to enrich the medium. Mother tank was placed in a dark room at 25±2ºC and 80% humidity for an acclimatization period of 2 weeks. During the acclimatization period regularly cow dung was added because it was a food for earthworms.

Identification and Characterization of the efficient earthworm vermicompost bacteria

Morphological Characterization

Morphological characteristics such as abundance of growth, pigmentation, optical characteristics, size and shape were studied on nutrient agar plates. Identification of bacteria was based on the examination of slides of Gram stained microorganisms prepared from pure cultures grown on agar agar.

Gram's Staining

The isolate was smeared on the glass slide and heat fixed. The crystal violet dye was added, kept for one minute and washed in running tap water. Then the gram's iodine was added, kept for one minute and washed in running tap water. It was decolorized with ethanol and then counter stained with safranine and washed in running tap water. It was observed in compound microscope. The bacteria that retained the crystal violet strain (appear violet) was designated as gram positive (G^+) and those cells that stained with pink color are called gram negative (G^-).

Spore Staining (Schaeffer-Fulton Method)

The isolate was smeared in the slide and heat fixed. Then the slide was flooded with malachite green and stream heat the slide for 2-3 minutes. Cool the slide and washed in running tap water. It was then added with counter stain safranin and kept for 30 seconds. Wash the slide with running tap water. Air dried the slide and examined under oil immersion, in microscope. The spores appeared green in color while the vegetative cells appeared red in color.

Biochemical Characterization

Indole Production Test

Indole production test is used to test whether the organism can have the ability to produce indole. Peptone broth was prepared, sterilized and cooled. Inoculate the test organism to the sterile peptone broth and incubate the tubes at 37°C for 24 hrs. The culture producing the cherry red color ring following the addition of kovac's reagent indicated as positive. The absence of red coloration indicated a negative result.

Methyl Red Test

Methyl red test is 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 incubate the tubes for 24 to 48 hrs 37°C. After incubation, add 7-8 drops of methyl red indicator and appearance of red color indicated the positive result.

Voges-Proskauer Test

This test is also known as the acetoin production test. This test is 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 incubate 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 is 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.

Triple Sugar Iron Test

TSI test is used to differentiate the isolate according to the ability to ferment lactose, sucrose and glucose and production of hydrogen sulfide. Triple sugar iron agar medium was prepared and sterilized. Kept the tubes as slant and butt and allow it to solidify. Streak a loop full of test organisms on the surface of the slant and incubate at 37°C for 24 hrs. Acidification of the medium caused by the isolates attacking one of the sugars causes the phenol red indicator to change to yellow color. Bubble formation in the butt indicates the gas formation. Hydrogen sulphide production causes the formation of a black precipitate at the junction between the slope and the butt.

Nitrate Reduction Test

This test is used to detect whether the organisms reduced the nitrates to nitrites or not. Nitrate broth was prepared and sterilized. Inoculate one loop full of test culture and incubate at 37°C for 96 hrs. Following incubation, add 0.1 ml of test reagent (Sulphanilic acid and α-napthalamine) to the test culture. A red color developing within a few minutes the presence of nitrites and hence the ability of the organisms to reduce nitrates.

Result

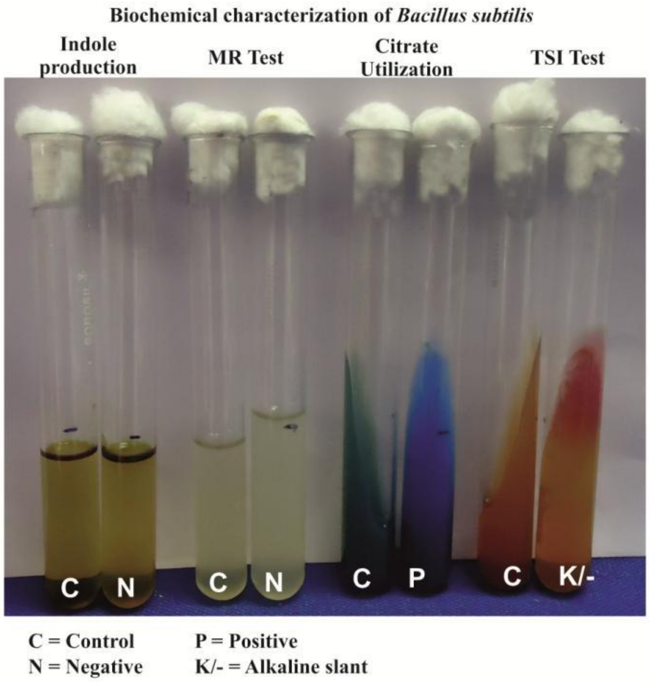
Table: 1 Biochemical Test for the identification of Vermicompost bacteria

Biochemical Test	***Bacillus subtilus***
Indole test	–
Methyl test	–
Voges Proskaver	–
Simmon Citrate	+
Nitrate reduction test	–
Catalase test	+

+ Positive; - Negative

Table: 2 Morphological Characteristics of isolated vermicompost bacteria

Name of the Organism	Staining	Shape
Bacillus subtilus	G^+	Rod



Discussion

Vermicompoating is a ideal process for municipal solid waste recycling and it will occur in a short period. It is easy method to carryout and it do not have any harmful effects. This type composting fully ecofriendly to vicinity. Pure microbial composting have a lot of risks but in vermicomposting there is no risks it is easy to do. It has a huge benficial effects to soil vicinity. 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; Singh and Kulbaivab 2009).

Table 2 shows the morphological characteristics of isolated *E. eugeniae* vermicompost bacterial species. *Bacillus subtilus* is an isolated bacterium. It is gram positive nature and rod in shape.

Table 1 contains biochemical results of *Bacillus subtilus* isolated from vermicompost of earthworm (*Eudrilus eugeniae*) under the long term study (28 days).

Several findings showed considerable increase in total viable counts of bacteria in the worm treated compost (Parthasarathi and Ranganathan 1998; Haritha Devi *et al* 2009). Teotia 1950 and also Parle 1963 isolated bacterial count of 32 million per gram in fresh vermicast. Scheu 1987 find out the increase of 90% in respiration rate in vermicast indicating corresponding increase in the microbial load. Vermicomposting alter the normal microbial community of the waste in a diverse way.

Our research was supported by Vaz-Moreira *et al* 2008; Blanc *et al* 1997. Vaz-Moreira *et al* 2008 isolated the *Bacillus species namely, B. cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis, B. macroides* from vermicomposts. Blanc *et al* 1997 also reported *Bacillus sp* from hot compost.

Conclusion

The awareness about organic fertilizers just like compost, vermicompost and vermiwash usage among farmers is emerged in recent years for produce safe agricultural products. Organic fertilizers usage awareness is to create the deficiency to adjust pesticides, insecticides related problem we give a 100% adopt solution that is municipal solid waste to convert into compost through earthworms and microorganisms.

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