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Diversity and quantitative study of bacteria of vermiwash samples

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ABSTRACT

Vermiwash is coelomic fluid and vermicasting filtrate due to earthworms (*Eudrillus eugenie)* activity, collected through vermiwash model. For investigation of bacterial diversity and its quantity four different samples of vermiwash used. The samples are VM1- of Horse, VM2- of Pig, VM3 - of Elephant and VM4 - of Cow dung. The bacterial strains are isolated from above samples by using serial dilution, screening and spread plate method of Jensen's, CRYMA and Nutrient agar medium. They are characterized and identified by observing morphological characters, microscopic examination and enzymatic activity. Purification of colony done by streak plate and slant streaking method. The bacterial strains identified are: From VM1- sample *Pseudomonas aeroginosa* ,VM2- *Rhizobium spp.* and *Azobactor spp.*, VM3- *Pseudomonas aeroginosa* and VM4- sample *Rhizobium spp. and Azobactor spp.* respectively. The maximum bacterial count VM1- sample- 42 X 10^3 SPC/ ml, VM2- sample - 33 X 10^1 SPC/ ml, VM3- sample - 76 X 10^1 SPC/ ml, VM4- sample- 54 X 10^2 SPC/ ml. The bacterial flora in vermiwash very useful to regulate growth of plants with respect to nitrogen fixation and white root proliferation.

Introduction

Earthworms are biological indicators of soil fertility and supports healthy population of bacteria, fungi, actinomycetes, protozoans, insects and millipedes, are essential for sustaining a healthy soil. An earthworm not only inhabits the soil but by virtue of their activity contribute physical and chemical alternation in the soil but leading to soil fertility and plant growth (Barley, 1959). Millions of years before these silent biological machines have been performing a marvellous function of ploughing the soil and fertilizing them (Bhatia, et al. 2001). Vermiwash is nothing but coelomic fluid and vermicasting filtrate. Earthworms and their by-products play sustainable role in organic farming for betterment of human being and ecosystem at large. Vermiwash acts as organic liquid bio- fertilizer as it contains different micronutrient, enzymes, growth regulators and microbes (Patil, et al. 2007). The microbes play vital role in transforming- Nitrogen, Phosphors, Potassium and Sulphur also the micronutrient like Fe, Cu, Zn, Mg and Mn in utilizable form (Gish, et al. 1973). In this way microbes play important role in different physiological and bio-chemical capabilities could be introduced into root zone of plants in consortium microbes are used *Acetobacter diazotrophicus* as nitrogen fixer, *Bacillus subtilis* as phospahate, potassium and silicon solubilizers, *Thiobacillus* bacteria are sulphur oxidises and *Lactobacillus* bacteria are photosynthetic (Parle, 1963, a and b). Earthworms has efficiency to consume all types of organic waste including agricultural, agro-industrial, vegetable waste respectively. Earthworms not only inhabits in the soil but by virtue of their activity contributes physical and chemical alternations, where soil fertility increases and turns richly inhabited by micro-organisms. Microbes in the environment significantly influence the biogeochemical cycle of phosphorus. The organic phosphorus compounds are decomposed and mineralized by enzymatic complexes like phosphatases produced by microbes. In the ecosystem to promote enzymatic degradation of naturally occurring phosphorus compounds (Gavrilov, 1973).

Materials

Vermiwash samples obtained by using earthworm species *Eudrillus eugenae* fed on different food sources of horse, pig, elephant and cow dung. The different medium used for cultivation of bacteria are Nutrient agar medium, Jensen's medium and Congo red yeast extract mannitol agar (CRYMA). The reagents and chemicals are alcohol, grams staining reagent and distilled water. Glass wares and instruments are test tubes, pipettes (1ml, 2ml, 5ml and 10ml), petri plates, incubator, refrigerator, microscope, colony counter and autoclave.

Methodology

Collection and screening of different vermiwash samples. Isolation of micro-organisms from above samples by using serial dilution and spread plate technique. The different medium used for cultivation of bacteria are Nutrient agar medium, Jensen's medium and Congo red yeast extract mannitol agar (CRYMA). Determination of standard plate count (SPC) of different vermiwash samples by using formula:

Standard Plate Count (SPC) = Number of colony X **Dilution factor** X **10**

Identification and characterization of isolated micro-organisms by morphological characteristics of colonies, microscopic examination, gram nature, motility and enzymatic or biochemical properties. Purification of colony done by streak plate and slant streaking method. The maintenance of isolated cultures were maintained on selective media at 4^{O}C in refrigerator.

Results

The bacterial isolates were obtained from 4- samples of vermiwash on selective medias i.e. Nutrient agar medium, Congo red yeast extract mannitol agar (CRYMA) and Jensen's

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medium. The three isolates obtained were studied for their cultural, morphological and biolchemical characterstics and were tentative identified as *Azotobactor spp.*, *Rhizobium ssp.*, *Pseudomonas aeroginosa.* Vermiwash sample VM2, VM4 from earthworms fed on cow and elephant dung as food shown the growth of *Azobactor ssp.* As well as *Rhizobium ssp.* Bacterial colony in vermiwash sample VM1 and VM3 shows growth of *Pseudomonas aeroginosa* and maximum growth of microorganisms shown in the VM1 sample i.e. 42 X 10^3 SPC/ ml of bacteria on Nutrient agar medium (Ref. Table- 1 and Table- 2)

Table 1: The SPC/ ml of bacteria from different vermiwash samples on various agar media

Sample	SPC/ ml of bacteria on Nutrient agar medium	SPC/ ml of bacteria on Jenson's medium	SPC/ ml of bacteria on CRYMA medium
VM-1	42 X 10^3	34 X 10^3	38 X 10^2
VM-2	30 X 10^1	32 X 10^1	33 X 10^1
VM-3	76 X 10^1	29 X 10^2	36 X 10^1
VM-4	40 X 10^2	54 X 10^2	44 X 10^3

Table 2: The bacterial micro-organisms of various vermiwash samples

Sample	Growth of Micro- organism
VM-1	*Pseudomonas aeroginosa*
VM-2	*Rhizobium ssp.* and *Azotobactor spp.*
VM-3	*Pseudomonas aeroginosa*
VM-4	*Azotobactor spp.* and *Rhizobium ssp.*

Discussion

The present study was carried out to evaluate composition of vermiwash by considering biochemical and microbial approaches for sustainable development of plant growth promoting factors and as a soil conditioning agent. In vermiwash urease producing microbes were found, which converts red coloured urea agar in to pink colour. In this way microflora of vermiwash is studied which shows presence of *Azobactor ssp.*, *Rhizobium ssp.* and *Pseudomonas aeroginosa.* Presence of these microbes makes available inorganic nitrogen, amino acids and inorganic phosphate to the plants through aminofication and nitrification process. Vermiwash contains mainly earthworm excreta and decomposed matter in liquid form. As the main substrate present in the waste, which is rich source of macromolecules. Resultant complex materials can easily break with enzymes secreted by earthworms. Soil with similar substances are the best and suitable media for growth of various micro-organisms.

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