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A study on isolation and characterization of Earthworm gut flora of *Perionyx excavates*

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ABSTRACT

From the Earthworms (*Perionyx excavates*) gut the bacterial and fungal isolates were selected and identified using various biochemical tests. The bacterial isolates such as *Bacillus* sp, *Escherichia coli, Micrococcus* sp, *Pseudomonas* sp, *Cellulomonas* sp and the fungal isolates such as *Aspergillus niger, Mucor* sp, *Penicillium* sp and *Rhizopus* sp were identified. All the identified isolates were selected for characterization studies such cellulolytic, lignolytic and phosphate solubilization. Among the isolates *Bacillus* sp, *Cellulomonas* sp, and *Aspergillus niger* were found to degrade cellulose. In addition to cellulose degradation Aspergillus niger was found to degrade lignin and none of the bacteria responded to lignin. On the other hand Pseudomonas sp and Bacillus sp were found to solubilize tricalcium phosphate effectively.

Key words: Earthworms, *Perionyz excavates*, microbes.

INTRODUCTION

Earthworms are soil invertebrates that play a key role in recycling organic matter in soils. They are also called as "Ecocystem Engineers" (Satchell, 1967). An earthworm improves the soil mix by helping it to achieve the proper air, water and increases the soils ability to absorb water. Earthworm while ingest organic waste and soil, consume heavy metals through their intestine as well as through their skin. Since the intestines of earthworms harbour wide ranges of microorganisms, enzymes, hormones etc., these half digested material decompose rapidly and are transformed into a form of Vermicompost (Edwards, 1972). The bacterial species belonging to the genera Bacillus and Pseudomonas species possess the ability to bring insoluble phosphates in soil into soluble forms by secreting organic acids like formic, acetic, propionic, lactic, glycolic, fumaric, and succinic acids (Sathe, 2004). Cellulose is the most abundant chemical constituent of a cell. The degradation of cellulose is catalyzed by celluloses. Several varieties of fungi, including species of the Genera Aspergillus, Fusarium, Trichoderma and bacteria including Vibrio, Cellulomonas, Polyangia, Cytophaga, Streptomyces and Nocardia, exhibit significant cellulolytic activities (Imshenetsky 1967, Ljungdahl and Eriksson, 1985).Krause et al., 2003; Malherbe and Cloete 2003, described that the lignolytic enzymes are widely considered to play a key role in the enzymatic degradation and it includes phenol oxidase, Laccase, peroxidases, Lignin Peroxidases (Lip) and Manganese peroxidase (Mnp). Fungi breakdown lignin aerobically through the use of a family of extra cellular enzymes collectively known as "Lignases".

MATERIAL AND METHODS

Gut analysis

The earthworms (Perionyx excavatus) were collected and washed with sterile water to remove the surface microbial flora from its outer skin. The earthworms were then sacrificed by freezing and their whole body was dissected. The gut contents were taken for analysis and all plating works for bacteriological analysis were carried out immediately.

Isolation of earthworm gut microflora

For the fungal isolates, the colonies were identified by colony morphology and lacto phenol cotton blue staining. For bacterial isolates Gram staining, motility test and biochemical tests were performed and the bacterial strains were identified using Bergey's manual of determinative bacteriology (9th edition, eds. Buchanan and Gibbons)

Characterization of the isolates

Cellulose degrading character of the isolates

A piece of sterile filter paper was transferred aseptically onto the surface of mineral salt agar plate. The isolates were inoculated and incubated at 30^0 C for 1-2 weeks. The plates were observed at intervals during the incubation period for the appearance of brown color in the filter paper.

Lignin degrading character of the isolates

Malt extract agar medium (bacteria) and Meera Kumari *et al*., (2001) medium (fungi) were prepared and inoculated with the bacterial and fungal isolates. All the plates were incubated at 37^0 c for 24-48 hours.

Phosphate solubilizing character of the isolates

Pikovskaya's medium was prepared and inoculated (spotted) with the test organism. It was then incubated at 31^0 C for 7 days. After incubation it was observed for the clear zone around the phosphate solubilizers.

RESULTS AND DISCUSSION

Earthworms were collected from areas in and around Gandhigram and identified as Perionyx excavatus. Based on various biochemical tests the gut micro flora were identified as *Bacillus* sp, *Escherichia coli*, *Micrococcus* sp, *Pseudomonas* sp, *Cellulomonas* sp, *Aspergillus niger*, *Penicillium* sp, *Mucor* sp and *Rhizopus* sp.

Cellulose degrading efficiency of the isolates

Imshenetsky (1967) reported that in soil several varieties of fungi, including species of the genera Aspergillus, Trichoderma and bacteria including members of the genera Vibrio, Cellulomonas exhibit significant cellulolytic activities. The bacterial and fungal isolates were inoculated on Czapek's mineral salt medium with filter paper and incubated at 37°c for bacteria and 28°c for fungal growth. Among the isolates *Bacillus* sp, *Pseudomonas* sp, *Cellulomonas* sp and *Aspergillus niger* were found to degrade cellulose in faster rate than the other isolates.(Table 1).

Lignin degrading efficiency of the isolates

Kale (1991) studied the lignolytic organisms in earthworm worked soils and showed that symbiotic microflora of worms are involved in

Table 1: Cellulose degrading efficiency of the selected isolates

Organism	Culture growth	Color change of the filter paper	Cellulose degrading ability
Bacillus sp	Good growth	Brown	Good
Pseudomonas sp	Good growth	Brown	Good
Cellulomonas sp	Good growth	Brown	Good
Aspergillus sp	Good growth	Brown	Good
Escherichia coli	No Growth	No color change	Nil
Micrococcus sp	No Growth	No color change	Nil
Mucor sp	No Growth	No color change	Nil
Penicillium sp	No Growth	No color change	Nil

Table 2: Lignin and Phosphate degrading efficiency of the isolates

Organism	Lignin Degrading activity	Phosphate Solubilization activity
Bacillus sp	-	++
Escherichia coli	-	-
Micrococcus sp	-	-
Pseudomonas sp	-	++
Cellulomonas sp	-	-
Aspergillus sp	++	+
Rhizopus sp	-	-
Mucor sp	-	-
Penicillium sp	+	+

The symbols -, +, ++ indicates no growth, mild growth, good growth respectively.

lignin degradation. The bacterial and fungal isolates were screened for lignolytic activity using Crawford and Meerakumari medium incorporated with 1% tannic acid as a lignin source. Among the organisms tested Asperillus niger showed the better lignolytic activity by forming clearing zone with good growth in medium containing tannic acid.

Phosphate solubilizing efficiency of the isolates

Subba Rao (1982), reported that the soil microorganisms effectively release phosphorous from inorganic phosphorous through solubilization. All the isolates were characterized for phosphate solubilization using Pikovskaya's medium incorporated with 1%TCP. Among the isolates Bacillus sp, Pseudomonas sp, Aspergillus sp and Penicillium sp were found to solubilize TCP. (Table 2).

CONCLUSION

In this present study it can be concluded that the bacterial isolate such as Bacillus sp, Pseudomonas sp, Cellulomonas sp, and the fungal isolates such as Aspergillus niger can be effectively used for the degradation of lignin, cellulose and for phosphate solublization.

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