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ACTINOMYCETES FROM VERMICOMPOST AS POTENTIAL SOURCE FOR BIOACTIVE COMPOUNDS

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Abstract

Actinomycetes attribute to plant growth promotion as well as bio control abilities leading to increased agricultural yields and food safety. Earthworm castings present in vermicompost can be an imperative resource for bio prospecting novel actinomycetes. In the present investigation vermicompost was prepared using different organic substrates like *Arachis hypogeal* shell, *Cocos nucifera* mesocarp, *Oryza sativa* husk, *Calophyllum inophyllum* seed coat and Saw dust. Actinomycetes were isolated using Standard plate count (SPC) technique and identified based on colony morphology and microscopic appearance. The isolates obtained were screened for their inhibitory activity against human and plant pathogenic organisms by agar plug method. Amongst 50 isolates screened, GNSVA4 showed good antifungal activity. Molecular characterization of the isolate GNSVA4, showing good inhibitory activity was done using 16s RNA technique. This suggests that microbes established from vermicompost exhibit antimicrobial activity which can be used for suppression of pathogens in soil, further increasing the crop yield and improving human health.

Keywords:

Vermicompost,

Actinomycetes,

Antimicrobial activity

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1. Introduction

Vermicompost involves stabilization and bio-oxidation of primal material through the synergy of earthworms and microorganisms. Although microorganisms are mainly responsible for the biological decomposition of organic matter, earthworms play an imperative role in the process of fragmenting and conditioning the substrate, increasing the surface area for the growth of microorganism and altering its biological activity [1], [2]. Various biodegradable substrates like cow dung, agricultural residues, industrial wastes etc., are used for vermiculture. Substrates especially agricultural residues have been tested extensively in combination with an easily biodegradable substrate such as cow dung [3]. *Eudrilus eugeniae* is a prolific breeder, exhibits high reproductive rate, are ferocious feeder and survives under aberrant weather condition [4], [5].

Compared to soil the number of bacteria and actinomycetes in the gut of earthworms are present to a greater extent following an exponential law [6], [7]. The word "Actinomycetes" is derived from Greek word "atkis" (a ray) and "mykes" (fungus), having characteristics of both bacteria and fungi [8] but yet possess sufficient peculiar characteristic to demarcate them into kingdom bacteria. Actinomycetes are aerobic spore forming gram-positive bacteria; containing high G-C content (57-75%) in their genome, and belong to the order Actinomycetales characterized with substrate and aerial mycelium growth. They are prolific producers of several agriculturally important secondary metabolites and play a vital role in plant growth promotion as well as biocontrol abilities leading to increased agricultural yields [9], [10], [11], [12], [13]. *Streptomyces*, a gram-positive, antibiotics producer [14] plays a major role in recycling of organic matter. In the present investigation vermicompost prepared using different organic substrates was used for isolation and identification of actinomycetes. The isolates were then screened for their antimicrobial activity against a few human and plant pathogenic microorganisms. Molecular characterization of the most potent isolate showing good inhibitory activity was done using 16S rRNA technique.

2. Research Method

2.1. Preparation of vermicompost:

Organic substrates like *Arachis hypogeal* shell, *Calophyllum inophyllum* seed coat, *Cocos nucifera mesocarp*, *Oryza sativa* husk, Saw dust, bulking agents (kitchen waste and garden waste) were collected and air dried. As per requirement chopping and shredding was done to speed up decomposition and hasten the process of composting by increasing the surface area for microbial action and providing better aeration. Five plastic tubs of 25 Kg capacity were used for preparation of vermicompost. The organic substrates were mixed with the bulking agents in 1:1 ratio w/w making up to 10 Kg. Cow dung slurry was added to each tub, mixed properly and allowed to decompose partially for three to four weeks [15], [16], [17], [18]. During this time proper turning was given to cool the substrates as well as to get homogenous compost. Water was sprinkled ad libitum to maintain moisture level between 40-60% [19]. Earthworms (*Eudrilus eugeniae*) were released at 50 numbers/10 kg of mixture and kept in shade for three to four months. By this process, earthworms multiplied 300 times within one to two months until the organic material was completely biodegraded. After complete decomposition of organic matter the vermicompost was harvested and air dried in shade. The obtained vermicompost was stored in storage bottles and used for further study.

2.2. Isolation and identification of Actinomycetes:

The isolation of actinomycetes was carried out by serial dilution of the vermicompost. One gram of the vermicompost sample was diluted serially upto 10-4, the dilutions were plated on Starch Casein Agar (SCA), by spread plate technique and incubated at 27 ± 2 0C for 5-7 days. The most prominent colonies were isolated and maintained on SCA slants at 40C for further studies.

2.3. Antimicrobial activity of the actinomycetes isolates:

The antibacterial and antifungal activities of actinomycetes isolates were evaluated by the agar plug method. Briefly, actinomycetes isolates were screened for their antimicrobial activity against three human pathogens and seven plant pathogens. The human pathogens tested were Gram positive bacteria (ATCC29213, *Staphylococcus aureus*) and Gram negative bacteria (ATCC25922, *E. coli* and ATCC27853, *Pseudomonas aeruginosa*). Plant pathogens tested were two Gram negative bacteria (*Ralstonia solanacearum* and *Xanthomonas oryzae*) and five fungal plant pathogens (*Alternaria helianthi*, *Aspergillus niger*, *Colletrotricum capsici*, *Mangoporthe grisea*, and *Scleroritium rolfsii*). Bacterial cell suspension and fungal spore suspension were swabbed on modified nutrient glucose agar (MNGA) medium plate. The actinomycete isolates plugs were cut by the cork borer and placed on the surface of MNGA medium seeded with the test organisms. The bacterial and fungal plates were incubated at 37^0C for 24h and 27^0C for 48 h respectively. The presence of bioactive compound was determined by observing the zone of inhibition around the actinomycetes plug. The results were recorded by measuring the zone of inhibition in diameter (mm).

2.4. 16S rRNA sequencing and phylogenetic analysis:

One of the most potent actinomycetes isolate labeled as GNSVA4, which showed good inhibitory activity against many fungal pathogens was selected for further studies. The Genomic DNA was isolated and quantity was measured using Nano drop spectrophotometer and the quality was determined using 2% agarose gel. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.

Consensus sequence of 16S rRNA gene was generated from forward and reverse sequence; 16S rRNA gene sequence was used to carry out BLAST with the database of NCBI gene bank database. Based on maximum identity score, first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed by using MEGA 7.

2.5. Statistical analysis: The data collected were subjected to statistical analysis following Duncan's Multiple Range test (DMRT) and one way ANOVA.

3. Results and Analysis

3.1. Isolation and identification of Actinomycetes:

A total of 50 actinomycetes isolates were identified and isolated from the vermicompost prepared using five different organic substrates (*Arachis hypogeal shell, Calophyllum inophyllum* seed coat, *Cocos nucifera* mesocarp, *Oryza sativa* husk and Saw dust). Initially, the colonies are relatively smooth surfaced, but later they develop a tuft of aerial mycelia that may appear floccose, granular, powdery, or velvety. Most of the colonies

formed were hard, leathery or fluffy round colonies with discrete edges and microscopically they appeared as Gram positive rods in filament. [20], [21].

3.2. Antimicrobial activity of the actinomycetes isolates:

Amongst the 50 isolates screened, 10 isolates showed antibacterial activity and four isolates exhibited good antifungal activity. The zone of inhibition was maximum against fungal pathogens compared to tested bacterial pathogens. One of the isolate GNSVA4 showed broad spectrum inhibitor activities against many fungal pathogens and the same was selected for further morphological characterization. GNSVA4, was isolated from *Arachis hypogeal* shell vermicompost. GNSVA4, showed significantly maximum zone of inhibition against *Colletrotricum capsici* (22 mm) followed by *Aspergillus niger* and *Scleroritium rolfsii* with 20 mm and 13 mm respectively "Table 1". Actinomycetes can defend the fungal pathogens by production of certain bioactive compounds and safe guard the roots, in turn increasing the crop yield [22], [23], [24]. Agricultural association of these beneficial organisms in biocontrol of plant pathogens is reported earlier [25], [26], [27]. These organisms can trigger signal transduction in host plants to initiate defence responses to cope with the stresses at cell, tissue and organ level following inoculation of these organisms [28]. Actinomycetes are promising source of many biologically active compounds [29], [30], [31], [32] which have important applications in human medicine and plant growth. According to Pan *etal;* bafilomycins B1 and C1 produced by *S. cavourensis* NA4 could be two promising lead compounds for the development of a potential biocontrol agent (fungicides) for soil borne fungal diseases of plants [33].

Table 1. Antifungal activities of actinomycete isolate (GNSVA4).

Fungal plant pathogens	Zone of inhibition in diameter (mm)
Aspergillus niger	20^{b}
Colletrotricum capsici	22^{a}
Scleroritium rolfsii	13^{c}

3.3. 16S rDNA sequencing and phylogenetic analysis:

16S rRNA gene was amplified by 16S rRNA F and 16S rRNA R primers. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose gel "Fig. 1".The PCR amplicon was purified to remove contaminants.

The evolutionary history was inferred using the Neighbor-Joining method [34]. The optimal tree with the sum of branch length = 4.11859794 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches [35]. The evolutionary distances were computed using the Maximum Composite Likelihood method [36] and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 794 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [37].

Actinomycete isolate labeled as GNSVA4, showed 99% similarity with *Streptomyces cavourensis* strain producing significant alignments from BLAST "Fig. 2".

igure 1: A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose gel.

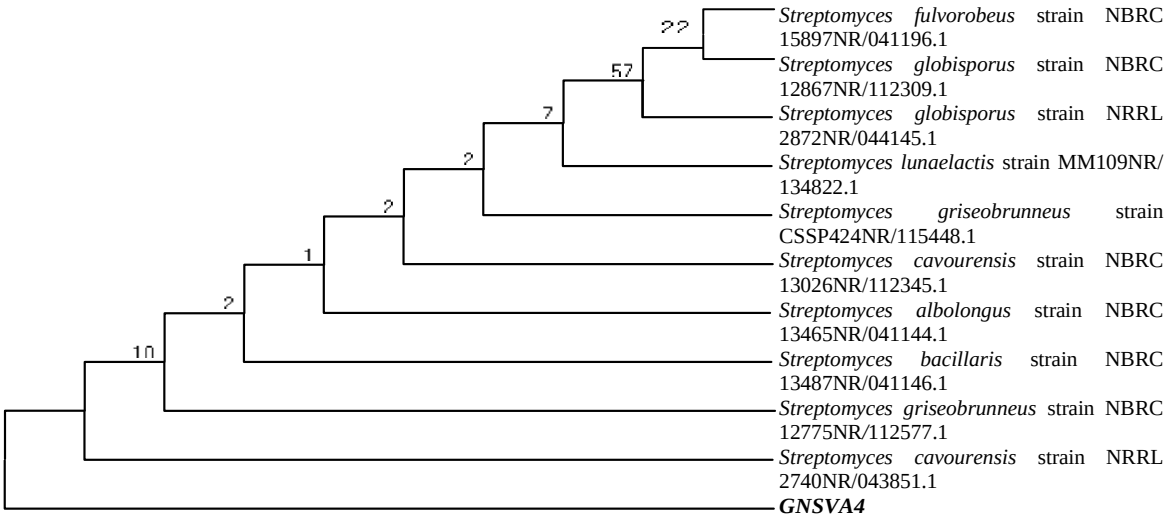

Figure2*: Evolutionary relationships of taxa*

4. Conclusion

The recent interest is to develop healthy crops from healthy soil. This is the need of the day to get good food to restore the health of the population. In this regard one has to take up the chemical free agriculture. By enhancing the levels of beneficial organisms in the soil, it is possible to curb the growth of plant pathogens in the soil environment. By developing beneficial organisms that can contribute to release of bio agents that can enrich the soil through appropriate mode of composting methods, it is possible to enhance the soil health through application of quality organic inputs.

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