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A novel of bioprospecting of earthworm cast associated actinobacteria for its antimicrobial properties--Short communication

Gopikrishnan, V.[1*], Radhakrishnan, M. [1], Joseph, J. [1], Abirami, B.[1], Manigundan, K.[1] , Suresh, A.[2] and Soytong, K.[3]

[1]Centre for Drug Discovery and Development, Dr. Col. Jeppiaar Research Park, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu - 600 119, India; [2]Central Research Laboratory, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research (MAHER), Enathur, Kanchipuram, India; [3]Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology, Landkrabang, Bangkok, Thailand.

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Abstract Among bacteria, the actinobacteria are the most significant source of bioactive compounds and many industrially important by products in use today. The present study designed to isolate the actinobacteria from earthworm cast for its antimicrobial properties. Totally 56 actinobacterial colonies were isolated from earthworm cast of Kanchipuram agricultural area. Of which, 36 morphologically different colonies were isolated and identified which mostly belong to *Streptomyces*. In antimicrobial activity, among 36 isolates, 12 were showed antimicrobial activity against one or more tested human pathogens eg *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. So, the study revealed earthworm cast associated actinobacteria can be recorded as an imperative resource for bio-prospecting that could produce novel bioactive metabolites.

Keywords: Actinobacteria, Earthworm cast, Streptomyces, Antimicrobial and bioprospecting

Introduction

Earthworms are large megadrile annelids of the class Oligochaeta that constitute more than 80 % of the soil invertebrate biomass in many terrestrial ecosystems (Nainawat and Nagendra, 2001). They form the major group of soil invertebrates in subtropical, tropical and temperate zones (Kale, 1997). Earthworms feed on soil and on a wide range of decaying organic substances, and excrete wastes in the form of casts. Earthworm casts consist of masses of

[*] **Corresponding Author:** Gopikrishnan, V.; **Email:** gopivktk@gmail.com

mineral soil often mixed with smallerbits of partially digested plant residues. Earthworm cast are bioorganic engineered structures build by earthworms through decaying soilorganic substances. It's a major environmental activity of earthworm cause significant impacton soil fertility and plant growth. Numerous microbes, enzymes and nutrient rich waste materials ofplant and animal present in casting, which are unable to digest by the earthworm. Earthworms associated micro-biomes are potential sources of high biotechnological interest because of its greatdiversity of compounds exhibiting a broad spectrum of biological activities (Malliga *et al.*, 2011). Kumar *et al.* (2012) reported that earthworm castings were isolated and found Actinomycetes that expressed antimicrobial activity and enzymes. The dominant species were Streptomyces and followed by Streptosporangium, Saccharopolyspora and Nocardia. Some isolates found to produceamylase, caseinase, cellulase, gelatinase, xylanase and lipase. Streptomyces rochei expressed a promising of antimicrobial activity.

To date, researchers focusing on novel microbes in unusual and unexplored ecosystems. Actinobacteria are unique secondary metabolites producers having versatile bioactive compounds with industrial importance. The earthworm cast are less explored for actinobacteria having bioactive compounds. This short study clearly says that the earthworms and its associated microbes can largely explored for bioprospecting, especially actinobacteria. By the way developing and conserving the earthworm associated microbial diversity is playing a major role in economic development of country.

The objectives of research project were collected and identified the earthworm cast soil samples from agricultural areas at Kanchipuram, Tamil Nadu, India and testing their antimicrobial activities against human pathogens, *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Materials and methods

Collection and isolation of actinobacteria

The soil samples were collected from agricultural area at Kanchipuram, Tamil Nadu. The samples were transported in a sterile polythene bag into the laboratory and dried at room temperature for 3 days. One gram cast sample was weighed and heat treated at 55℃ for 10minutes.One gram soil was suspended in 9ml of sterile distilled water. The samples were serially diluted up to 10^{2} dilution using 9ml sterile distilled water blanks. Hundred microlitre of aliquot from10^{3}, 10^{4} and 10^{5} dilutions was transferred to starch case in agar pH7

supplemented with nystatin and nalidixic acid and cellulose agar medium plates and was spreaded using sterile L-rod. The plates were incubated at room temperature for 1month. The actinobacterial colonies selected from isolation agar medium was streaked on ISP2 agar plates (yeast extract 4.0%; malt extract 10.0 %; dextrose 4.0%; pH7.0; agar 1.8%; distilled water 100ml) and incubated at 28℃ for 5 to 7 days. After incubation cultural characteristics such as aerial mass color, re-verse side pigment, soluble pigment and microscopic appearance such as the presence of substrate and aerial mycelium of all the actinobacterial isolates were detected by observing under bright field microscope with 40x magnifications (Malliga *et al*., 2011).

Screening of earthworm cast associated actinobacteria for antimicrobial activity

Antimicrobial activity of earthworm cast actinobacterial cultures were tested by adopting agar plug method (Malliga *et al.*, 2011). Actinobacterial cultures were grown on Yeast Extract Malt Extract (YEME agar) plates and were removed from the agar surface using sterile spatula. Tested pathogens (*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) with 0.5 McFarland standards were transferred into nutrient agar plates using sterile cotton swab. All the plates were incubated at 37℃ for 24 hours. Zone of inhibition was measured after incubation and expressed in millimetre in diameter according to the modified method of Eccleston *et al.* (2008).

Results

The cast material from the agricultural soil around Kanchipuram city was collected and actinobacteria were isolated using Nutrient, Starch casein and Cellulose agar media. After incubation, 56 morphological different bacterial colonies were isolated (Figure 1). Cultural, morphological and physiological characteristics were indicated that 36 isolates belonged to the genus *Streptomyces*. All the isolates showed good growth and some of them produced pigments on yeast extract malt extract agar medium (Figure 2). In antimicrobial activity test, out of 36, 12 isolates of *Streptomyces* were found to be actively antimicrobial activity against one or more tested human pathogens, *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) (Figure 3). It found the clear zone inhibition between isolates of *Streptomyces* and tested human pathogens.

Figure 1. Isolation of bacteria and actinobacteria from earthworm cast

Figure 2. Colony morphology of bacteria and actinobacteria

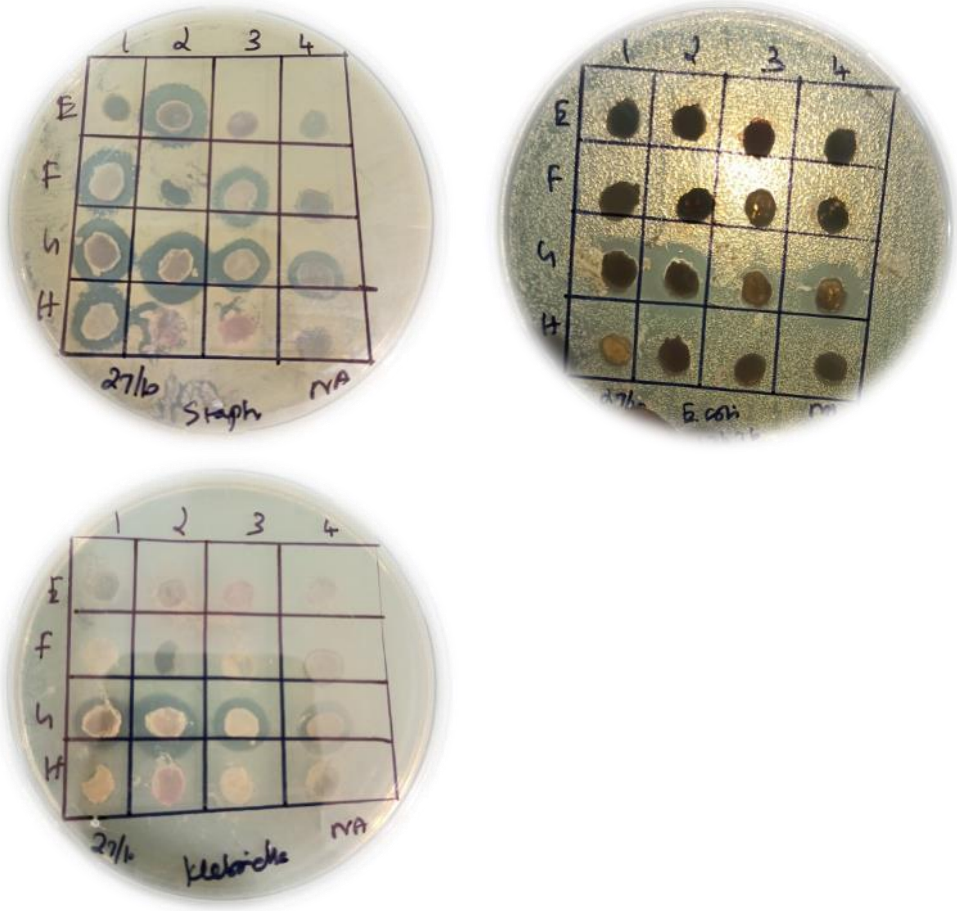

Figure 3. Antimicrobial activity of earthworm cast actinobacteria by agar plug method

Discussion

Though, there are good reports were available on isolation of actinobacteria from earthworm cast in agricultural soil, but its antimicrobial activity was less reported (Malliga *et al*., 2011; Kumar *et al*., 2012; Ruanpanun and Chamswarng, 2015). The study revealed that earthworm castings are potential source for a wide spectrum of antimicrobial producing actinobacteria, especially *Streptomyces*. Moreover, it can be an essential source for bio-prospecting novel/ rare actinobacteria, which could yield precious bioactive molecules.

Our research finding appeared 12 isolates of *Streptomyces* actively antimicrobial activity against one or more tested human pathogens, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as seen the clear zone inhibition. Withthis, Kumar *et al.* (2012) reported that the found isolates from earthworm cast were tested antimicrobial activities against human pathogens, *Staphylococcus aureus* MTCC2940, *Candida albicans* MTCC1637, *Microsporum canis* MTCC3070 and plant pathogen, *Macrophomina phaseolina*. As result, *Streptomyces* spp. actively dominant species for antimicrobial activites are preliminary recorded and further studies are being done in advances for their active metabolites and other reactions.

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