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Exploration of earthwormcast associated actinobacteria for plant growth promoting properties

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Abstract Due to their wide varieties of chemicals with a variety of biological actions, earthworms, cast, and gut are potential sources of significant biotechnological interest. The finding new microorganisms in peculiar and uncharted habitats were investigated. Actinobacteria are exceptional makers of secondary metabolites and have a wide range of bioactive substances of economic significance. In the current investigation, actinobacteria from earthworm casting were isolated for their potential antibacterial capabilities. Earthworm cast from the Kanchipuram agricultural area had a total of 12 actinobacterial colonies. Human and plant pathogens such as *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Ralstonia solanacearum* were tested against each actinobacterial cultures. The selected potential culture was examined for the presence of Indole Acetic Acid (IAA), Phosphate (P) solubilization, extracellular enzymes such as cellulase and amylase. For the promising active trains, optimization, characterization and taxonomy were carried out. Colonies with various morphologies were separated and subcultured for testing their activities. Six of the twelve isolates proved positive for antibacterial activity against one or more pathogens. Of the six isolates tested, strain EWCA 21 was chosen for further screening because it had the greatest effectiveness against tested pathogens. IAA, Phosphate (P) solubilization, and extracellular enzyme activities such amylase and cellulase were demonstrated in EWCA21. Carbon and nitrogen sources expressed an increase in activity and output during optimization. The putative strain was determined to be *Streptomyces* based on phenotypic analysis. The study concluded that the strain EWCA21 would be developed as a crucial resource for biofertilizer applications.

Keywords: Actinobacteria, Earthworm cast, Streptomyces, Antimicrobial and bio-fertilizer applications

Introduction

For many years, scientists have been developing microbiological tools, biofertilizers, and biocontrol agent's bacteria and fungi that can

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promote the health and growth of crop plants. Currently, a variety of strains based on soil, plant-growth-promoting rhizobacteria (PGPR), epiphytic fungi and mycorrhizal fungi are accessible as products for agriculture. Each of these strains faces unique production and use issues, with inconsistent field performance being the main issue. The demand for biofertilizers and biocontrol agents is anticipated to rise in response to the growing concern throughout the world about pollution, greenhouse gas accumulation and the need for more plant-based foods (Koskimaki *et al*., 2021). Actinobacteria are considered as the most prominent source of bioactive compounds (antibiotics, enzymes, and plant growth modulators) facilitating plant growth promotion and plant disease suppression. Attempts are being made to utilize actinobacteria that produce antibiotics and agro-active compounds as biofertilizers and biopesticides; these aids in mitigating the use of harmful chemical fertilizers and pesticides (Shivlata and Satyanarayana, 2017). The focus of research to far has been found to be the novel microorganisms in unusual and unexplored settings (Gopikrishnan *et al*., 2021).Earthworm casts are biologically constructed structures that earthworms construct using decomposing soil organic materials. Earthworm activity has a considerable negative influence on soil fertility and plant growth. Casting contains a wide variety of microorganisms, enzymes and nutrient-rich plant and animal waste products that the earthworm cannot digest. Due to the diversity of chemicals exhibiting a wide range of biological actions, the microbiomes associated with earthworms are likely to be of considerable biotechnological interest (Malliga *et al.,* 2011). Less research has been done on actinobacteria with bioactive chemicals in the cast of earthworms (Gopikrishnan *et al*., 2021). This study clearly stated that earthworms and their accompanying microbes, particularly actinobacteria, can be extensively studied for bioprospecting. The goal of the research project was to preliminary evaluate the biofertilizer characteristics of actinobacteria present in the earthworm cast soil samples collected from agricultural areas in Kanchipuram, Tamil Nadu, India.

Materials and methods

Collection and isolation of actinobacteria

Soil samples were obtained at Kanchipuram, Tamil Nadu, from an agricultural area. The samples were brought into the lab in a sterile polythene bag, where they were dried for three days at room temperature. One gramme of earthworm cast sample was weighed and heated for 10 minutes at 55^{o}C. In 9ml of sterile distilled water, one gramme of soil was suspended. Using 9ml sterile distilled water blanks, the samples were diluted serially up to 10^{2} - 10^{5} respectivelly. A 100 microliter aliquot from dilutions 10^{3}, 10^{4}, and 10^{5} was transferred to starch casein agar pH 7

medium plates supplemented with nystatin and nalidixic acid and was distributed using a sterile L-rod. The plates were incubated for a month at room temperature. The actinobacterial colonies that were chosen from the isolation agar medium were streaked on ISP2 agar plates (distilled water 100 ml, pH 7.0, agar 1.8%, yeast extract 4.0%, malt extract 10.0%, dextrose 4.0%, and agar 1.8%) and cultured at 28 ℃ for 5 to 7 days. After incubation, all actinobacterial isolates' cultural properties, such as aerial mass colour, reverse side pigment, soluble pigment, and microscopic appearance, such as the presence of substrate and aerial mycelium, were observed under a bright field microscope at 40X magnification (Malliga *et al.,* 2011).

Screening and production of bioactive metabolites from earthworm cast associated actinobacteria

By using the agar plug method, the antimicrobial activity of earthworm cast actinobacterial cultures were evaluated (Malliga *et al*., 2011). Actinobacterial cultures were cultivated on Yeast Extract Malt Extract (YEME agar) plates and scraped with a sterile spatula from the agar surface. A 5 mm diameter agar plug was cut from the ISP2 agar after the incubation time using a well cutter and it was then placed over a nutrient agar plate that had been swabbed with test Pathogens (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Ralstonia solanacearum*) with 0.5 McFarland standards. All transfeered plates were incubated at 37 ℃ for 24 hours. The zone of inhibition was measured after incubation and expressed in millimetres in diameter (Eccleston *et al*., 2008). Submerged and agar surface fermentation were used to develop bioactive metabolites from the prospective strain, which were then tested against *Ralstonia solanacearum* every 24 hours until the 12^{th} day of fermentation (Gopikrishnan *et al*., 2013).

Screening of earthworm cast associated actinobacteria for IAA, Phosphate solubilization and enzyme activity

Standard procedures were used to produce Indole Acetic Acid (IAA) and phosphate solubilization. Using the starch agar and skim milk agar mediums, the activities of the enzymes amylase and protease were measured (Manigundan *et al.,* 2022).

Medium optimization

The impact of media and cultural states chemical compositions were investigated on the synthesis of bioactive compounds, and the traditional

one factor at a time method was used with the other factors being left unaltered (Balagurunathan *et al.,* 2017).

Characterization and taxonomy

The microscopic, cultural, and physiological properties of strain EWCA 21 were investigated using a conventional approach (Shirling and Gottileb, 1966).

Results

Actinobacteria were isolated from the cast material from the agricultural soil surrounding Kanchipuram city using starch casein agar media. Twelve different morphological bacterial colonies were isolated during incubation. Twelve isolates' cultural, morphological, and physiological traits suggested they belonged to the genus Streptomyces. On yeast extract malt extract agar media, most of the isolates exhibited good growth and some of them even produced colours. Six isolates of Streptomyces were reported to have active antibiotic activity against one or more tested human pathogens, including *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Ralstonia solanacearum*, out of a total of 12. (Table 1, Figure 1).

Table 1. Screening of earthworm cast associated actinobacteria for antimicrobial activity

s.no	**Strain name**	**Zone of inhibition in mm**				
		S aureus	***B cereus***	***P aeruginosa***	***E coli***	***R solanacearum***
1	ECA2	-	-	-	-	-
2	EWCA3	14	-	-	-	-
3	EWCA4	18	-	-	-	-
4	EWCA5	-	-	-	-	-
5	EWCA7	-	12	-	10	11
6	EWCA10	-	-	-	-	-
7	EWCA11	10	-	-	-	-
8	EWCA21	12	16	-	15	15
9	EWCA22	-	-	-	-	12
10	EWCA23	-	-	-	-	-
11	EWCA24	-	-	-	-	-
12	EWCA25	-	-	-	-	-

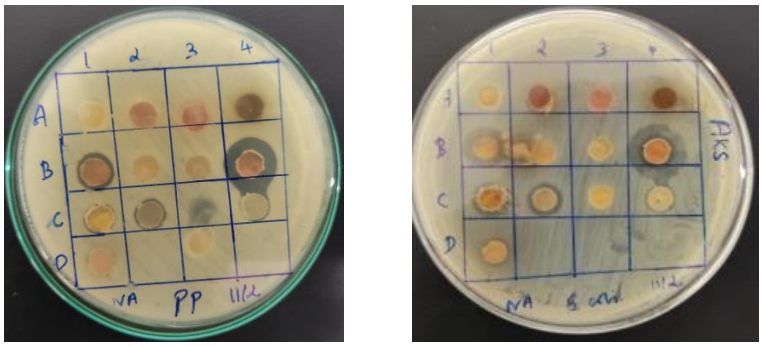

Figure 1. Screening of earthworm cast associated actinobacteria for antimicrobial activity

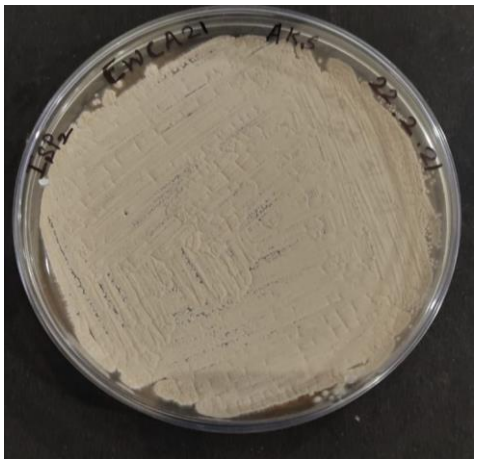

Figure 2. Potential strain EWCA21

Out of 6 isolates, strain EWCA21 demonstrated the greatest effectiveness against maximum of pathogens and was chosen as a viable strain for additional screening (Figure 2). Agar surface fermentation displayed good growth and encouraging activity to the end of the 12th day when compared to submerged fermentation. IAA, phosphate (P) solubilization, and extracellular enzyme activities such amylase and cellulose were demonstrated in EWCA21. The generations of bio-fertilizer as well as the activity of carbon and nitrogen sources were boosted during optimization. The possible strain was identified as belonging to the genus Streptomyces based on characteristics and taxonomy. Accordingly, the study found that the strain of actinobacteria linked with earthworm casts (EWCA21) will be a crucial resource for biofertilizer applications as well as for bio-prospecting that could provide novel bioactive compounds helpful for agricultural applications.

Discussion

Although there are several good publications on the isolation of actinobacteria from earthworm casts in agricultural soil, there are fewer reports on its antibacterial, IAA, and enzyme activity (Malliga *et al.,* 2011; Kumar *et al*., 2012; Ruanpanun and Chamswarng, 2015; Gopikrishnan *et al*., 2021). According to the study, earthworm castings are a possible source for a variety of actinobacteria that produce antimicrobials, including Streptomyces. It can also be a vital resource for bioprospecting novel or uncommon actinobacteria, which could produce valuable bioactive compounds. As observed by the clear zone inhibition, our research revealed that six isolates of Streptomyces had active antimicrobial activity against one or more tested human pathogens, including *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Ralstonia solanacearum*. EWCA 21 was chosen for future research because it shown promising efficacy against one or more pathogens tested. As a result, *Staphylococcus aureus* MTCC2940, *Candida albicans* MTCC1637, *Microsporum canis* MTCC3070, and *Macrophomina phaseolina* were examined for their antibacterial activity against the isolates discovered in earthworm cast, according to Kumar *et al.* (2012) and Gopikrishnan *et al.* (2021). The isolates from Kumar *et al*. (2012) also generated xylanase, lipase, and amylase enzymes. As a result, early records of the Streptomyces spp. actively dominant species for antibacterial activities have been made, and more research is being done on their active metabolites and other reactions. In earlier studies, we found that submerged fermentation on YEME broth produced the antifouling compound more slowly than agar surface fermentation on YEME agar. Cell-free supernatants from submerged cultures occasionally had little to no antibacterial action due to the chemical's diluted form, although the solvent extract of the same molecule did. A type of solid-state fermentation used to manufacture antifouling chemicals is agar surface fermentation (ASF), which is simple but less frequently used for metabolite production (Balagurunathan *et al*., 2016). In this article, we reported on the synthesis of antimicrobial metabolites by agar surface fermentation against *Ralstonia solanacearum.* Manigundan *et al*. (2022) reported *Streptomyces maritimus* from agricultural soil and its PGP properties like IAA, phosphate (P) solubilization, and extracellular enzyme activities. The traditional one element at a time method was especially employed to find the interaction of the significant components for boosting the synthesis of the antifouling compound because past studies showed that the optimization strategy maximizes the production of the antifouling compound (Balagurunathan *et*

al., 2016). Less information has been reported in the past on earthworm casts for PGP characteristics. The actinobacteria from earthworm cast have novel PGP characteristics, according to our reports. It is interesting to note that fewer studies have reported on actinobacteria associated with earthwormcast, despite the fact that many have detected antibacterial compounds from actinobacteria, including Streptomyces. In light of these studies' findings, it can be concluded that earthworm cast-associated organisms are promising candidates for use in sustainable agriculture management.

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