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ANTAGONISTIC EFFECT OF CERAMIC INDUSTRY SOIL STREPTOMYCESAGAINST PLANT PATHOGENS

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ABSTRACT

The various diseases that occur during the growth of plants usually cause a significant reduction in production and quality of agricultural products. Actinomycetes, especially *Streptomyces* spp., become a valuablebiological control resource due to their preponderant abilities to produce various secondary metabolites with novel structure and remarkable biological activity. The potassium solubilizing actinomycetes isolated from the ceramic soil industries were carried out anantagonistic effect against various soil borne phytopathogenic fungiand bacteria. Synthetic bactericides and fungicides have been causing harm to humans, animals, and the environment, as well as generating resistance in phytopathogenic organisms. Actinomycetes produce secondary metabolites with antifungal properties. Approximately 80% of antibiotics, such as streptomycin, spectinomycin, tetracycline and erythromycin, etc., are produced by actinomycetes. Organic acids produced by most of the actinomycetes inhibit the growth of phytopathogenic organisms and promote plant growth and induce systemic resistance in plants. Therefore, the objective of this study is to compare the antagonistic effect of actinomycetes isolated from different ceramic soil Industries and their application in agriculture. Although actinomycetes have equal or greater potential as biocontrol agents against phytopathogenic microbes.

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INTRODUCTION

Gram-positive aerobic bacteria, or actinomycetes, are widely distributed and exhibit a wide range of morphologies. Some of these bacteria, like those in the Streptomyces genus, have a resemblance to filamentous fungi (Ronald, 1997). These bacteria are ofbiotechnological interest because of their capacity to generate various bioactive chemicals. Actinomycetes have actually been the source of around two thirds of naturally occurring antibiotics (Buckingham, 1997; Newman et al., 2003). The agricultural and pharmaceutical industries find the microorganisms to be a desirable source of natural chemicals. As a result, efforts to screen microorganisms for bioactive compounds (enzymes and secondary metabolites) that are antagonistic to plant diseases have advanced. Numerous studies have examined the uses of microorganism as biocontrol agents in place of chemical fungicides in agriculture (Welbaum et al. 2004; Singh and Chhatpar 2011). Actinomycetes belonging to the Streptomyces genus are highly recognized for their capacity to impede an extensive variety of fungal phytopathogens, making them valuable microorganisms (Gomes et al. 2000). In this current research actinobacteria from soil and healthy plant tissues and screening them for in vitro antagonistic activity against bacterial and fungal phytopathogens, keeping in mind the significance of actinomycetes in biocontrol and plant growth promotion.

MATERIALS AND METHODS

Isolation of the Actinomycetes: Twenty-two strains of potassium-soluble actinomycetes were recovered from the ceramic industry soil in the Gujarat regions of Kadi, Meshana, and Morbi. Using Khandeparkar's selection ratio, a secondary screening process was conducted on the various isolates to determine their capacity for increased potassium solubilization. Standard culture, morphological, and analysis of the 16S rRNA gene sequence has been performed to select and identify the two actinomycetes strains, *Streptomyces fenghuangensis* (KSA 09) and *Streptomyces atacamensis* (KSA 16).

Methods Used in the Experiment: Actinomycetes from the KSA 09 and 16 strains were numbered, single colonies were chosen, purified, and cultivated for five days at room temperature. Using a sterile inoculation loop, pure colonies were obtained through peering at the actinomycete colonies' thin filaments under a light microscope. For further research, the isolated colonies were kept intact.

Selection of Pathogenic Bacteria and Fungi: Pathogenic bacterial strains, including *Pseudomonas*, *Xanthomonas*, and *Bacillus* species, were cultivated every 45 days on petri plates with nutritional agar at 4°C in a laboratory. Pathogenic fungus strains, including *Rhizopus* and *Fusarium* species, were cultivated every two months in a laboratory setting using petri plates with Potato dextrose agar (PDA) at 4°C. Subsequently, the promising isolates KSA 09 and 16 strains were introduced into PDA (Potato dextrose agar) medium for fungal cultures at room temperature and NA (Nutrient agar) media for bacterial cultures at 37°C. A subclass of prokaryotic organisms within the phylum Gram-positive bacteria are known as actinomycetes. The majority of them belong to the Actinomycetales order and subclass Actinobacteridae. Filamentous bacteria that belong to this order can create aerial and substrate mycelium, two different forms of branching mycelium. Their DNA has a high G+C concentration (>55 mol%), which sets them apart in part (Stackbrandt *et al.*, 1997). The interactions between rhizosphere actinomycetes that promotes plant growth and elicit defences against plant diseases are brought about by root exudates. Actinomycetes and plants communicate by exchanging nutrients to survive. Based on the amount of nutrients in the soil and the actinomycetes' capacity to communicate with the host plant, several kinds of interactions may form (Hassan *et al*., 2019).

A rhizospheric actinomycete isolate that was investigated by El-Sayed *et al*. (2023) had distinct and promising antagonistic activity against three of the most prevalent phytopathogenic fungi: *Alternaria brassicicola* CBS107, *Rhizoctonia solani* To18, and *Fusarium oxysporum* MH105. Based on spore shape and cell wall chemotype, the antagonistic strain was identified, and it was hypothesized that it is a member of the Nocardiopsaceae family. In addition, 16S rRNA gene phylogenetic analysis (OP869859.1) and cultural, physiological, and biochemical traits verified the strain's identity as *Nocardiopsis alba*. The strain's antifungal efficacy was assessed using its cell-free filtrate (CFF), and the tested fungal species showed inhibition zone widths ranging from 17.0 ± 0.92 to 19.5 ± 0.28 mm. Using a modified version of Dikin *et al*.'s protocol, the most powerful isolate was further evaluated using the dual culture plate assay against phytopathogenic fungal strains to confirm its antifungal activity (2006). Furthermore, it is one of the primary actinomycetes whose in vitro antifungal activity has been studied to suppress phytopathogenic fungi (Torres-Rodriguez *et al*., 2022). Examples of these include soil *Nocardiopsis dassonvillei*, which has been shown to suppress *Bipolaris sorokiniana* in wheat (Allali *et al*., 2019), airborne *Nocardiopsis alba*, which has been shown to suppress *Ganoderma boninense* (Widada *et al*., 2021), and soil *Nocardiopsis* sp., which has been shown to reduce *Fusarium* sp. (Adlin *et al*., 2019). Based on chemotaxonomy, phenotypic traits, and 16S rDNA sequence phylogenetic analysis, *Streptomyces flaveus* was determined to be the strain that suppressed all tested pathogens, SO1. According to Aquar *et al.* (2020), the results suggest that the SO1 strain has the potential to be used as a biocontrol agent and to boost plant growth. Using partial 16S rRNA sequencing, actinomycetes isolated from the rhizosphere soil were screened by Abdelrahman *et al.* (2022) for their ability to inhibit the growth of the fungal plant pathogen Phytophthora *infestans* through metabolic activity. Based on these results, several of the isolates were identified as *Nocardiopsis* spp.

Identification of Antagonistic Effect of Actinomycete Isolates: The cross-streak approach was utilized to identify the antagonistic effect against specific bacteria and fungi. For additional research, the pure KSA 09 & 16 strains with the strongest antibacterial activity were chosen. The cross-streak method was used to estimate the pathogenic bacteria and fungal inhibitory distance after they were incubated at 37 °C for 24 hours and 25 °C for 48 hours respectively, in order to assess the antimicrobial activity. The cross-streak method (Lemos *et al*., 1985) was used for primary screening against specific fungi and yeasts, and the agar cylinder method (Disk, 2007) was used for secondary screening against specific pathogenic bacteria (Taechowisan *et al*., 2005). For additional research, the isolates with the strongest antibacterial activity were chosen.

The formula for calculating the percentage of inhibition was (%) inhibition = (R control- R test)/R control x 100. where R control is the pathogenic fungus's colony diameter on SDA plates and R test is the pathogenic fungus's colony diameter on SDA plates with actinomycete isolates (Wang *et al*., 2002). The inhibition zone of inhibition's average diameter is used to classify the isolates' level of antibacterial activity. In this instance, the zone of inhibition's diameter was split into four categories: weak activity (≤9 mm), moderate activity (10–12 mm), outstanding activity (≥18 mm), and good activity (12–15 mm). Three separate samples were used. The ability of actinomycetes belonging to the genus *Streptomyces* to inhibit the growth of numerous fungal pathogens in vitro and in planta is widely recognized (El-Abyad *et al*., 1993; Abd-Allah, 2001; Coombs *et al*., 2004). The antagonistic activity of wheat (*Triticum aestivum* L.) rhizosphere isolates against specific root-rotting fungi (*Fusarium graminearum*, *Fusarium culmorum*, *Bipolaris sorokiniana* and *Fusarium verticilloides*,) were investigated. These are some of the action modes of the Streptomycetes in the rhizosphere. On solid media, the in vitro antagonistic effects of actinomycetes isolates against fungal pathogens were ascertained. Investigations were done on the mechanism of inhibition, the impact of application time, and pH on inhibition. With respect to all fungi, the actinomycete isolate 129.01 demonstrated a high inhibition ratio of greater than 60%. Under greenhouse circumstances, the isolate 129.01's ability to combat fungi that cause root rot was evaluated. 16S rRNA analysis supported the genus *Streptomyces* (Erginbase*t al*., 2010).

RESULTS

The findings showed that isolates KSA 09 had low antifungal activity but broad-spectrum activity against pathogenic bacteria (Gram-positive and Gram-negative), particularly against Pseudomonas spp. (Table 6.1). Most isolates grew best at a temperature of 35 to 37 0 C. Bacillus spp., (11.5 mm), Pseudomonas spp., strain 1 (13 mm), strain 2 (10.3 mm) and Xanthomonas spp., (12 mm) were the bacterial isolates that KSA 09 antagonistically affected, whereas KSA 16 in Bacillus spp., (6.5 mm), Pseudomonas spp. strain 1 &2) (8.5 & 6 mm), and Xanthomonas spp., (8 mm) were the isolates (Table 3. 1 and 3.2). The antagonistic research of KSA 09 and 16 against pathogenic fungal strains, including Fusarium spp. and Rhizopus spp., revealed that the former was less sensitive to KSA 09 than the latter to KSA 16. This finding verified that KSA 09, which is highly recommended, has an antagonistic effect on phytopathogens.

Table 1. Antagonistic Effect of Fungal Cultures with Zone of Inhibition

Fungi cultures	Zone of Inhibition (mm)	
	KSA 09	KSA 09
Aspergillus terreus	10.5	8
Rhizopus spp.	8.5	6.5

Table 2. Antagonistic Effect of Bacterial Cultures with Interpretation

Bacterial cultures	Zone of Inhibition (mm)	
	KSA 09	KSA 16
Xanthomonas spp.,	12	8
Pseudomonas spp. (St.1)	13	8.5
Pseudomonas spp. (St.2)	10.3	6
Bacillus spp.	11.5	6.5

Table . 3Antagonistic effect of Fungi cultures with Zone of Inhibition

Bacterial cultures	KSA 09	KSA 16
Xanthomonas spp.,	Moderate sensitive	Less sensitive
Pseudomonas spp. (St.1)	Highly sensitive	Less sensitive
Pseudomonas spp. (St.2)	Moderate sensitive	Less sensitive
Bacillus spp.	Moderate sensitive	Less sensitive

Table 4. Antagonistic effect of Fungi cultures with Interpretation

Fungi cultures	KSA 09	KSA 16
Aspergillus terreus	Moderate sensitive	Less sensitive
Rhizopus spp.	Moderate sensitive	Less sensitive

Actinomycetes, particularly *Streptomyces*, have been a subject of substantial study interest over the past 60 years due to their ability to create several physiologically active chemicals as part of this Gram-positive bacterial group. According to Silhavy *et al.* (2010), the isolates' crude extracts demonstrated antibacterial activity against Gram-negative bacteria, which are frequently more resistant to antimicrobial treatments than Gram-positive bacteria. Actinobacteria's antagonistic effects on pathogenic fungus have been evaluated across a wide range of fungal species. Various studies have demonstrated the capability of actinobacteria isolates to counteract phytopathogenic fungi (Aouar *et al*. 2012; Goudjal*et al*. 2016; Abbasi *et al*. 2019). Putrie*et al*. (2020) identified actinobacteria as the predominant microorganisms in the rhizospheric soil. Khamna *et al*. (2009) showed that microorganisms from the rhizosphere are best for controlling plant diseases, which is why we have studied rhizospheric soil. Actinomycete isolates were isolated and screened by Kaur *et al.* (2013) for potential antagonistic activity and activities that promote plant growth. Of the 321 isolates, 156 were rhizospheric, 103 were non-rhizospheric, and 62 were endophytic. Eighty-three isolates demonstrated antagonistic activity against one or more test phytopathogenic fungi during the dual culture assay primary screening. Out of the activeisolates, 20 showed antagonistic properties in the well diffusion experiment during the secondary screening. The majority of them exhibited a wide-ranging inhibitory effect against five to six test fungi. The study's findings suggest that these isolates have potential applications as biocontrol and plant growth boosting agents. *Streptomyces* was the genus that all of the active isolates belonged to, according to morphological and chemotaxonomic investigations. Considering the aforementioned results, the objective was to isolate actinomycetes with potent antibacterial properties from ceramic soils, which serve as a model for a harsh ecology. Out of the 22 actinomycetes found in ceramic soils, *Streptomyces fenghuangensis* (KSA 09) and *Streptomyces atacamensis* (KSA 16) showed wide-ranging phytopathogenic effects against various harmful bacteria and fungi. The KSA 09 and KSA 16 isolates were identified as *Streptomyces fenghuangensis* and *Streptomyces atacamensis* by a combination of conventional and molecular approaches.

CONCLUSION

An additional round of antimicrobial screening was performed on each isolate to check for harmful fungus and bacteria. According to the results, two of the isolates (KSA 09 and KSA 16) exhibited antagonistic effect against the majority of the pathogenic bacteria that were tested. Consequently, these two intriguing isolates were chosen for further identification after being previously determined to be Streptomyces via morphological, biochemical, and physiological techniques. The findings showed that isolate KSA 09 had modest antifungal activity but broad-spectrum action against pathogenic bacteria (Gram-positive and Gram-negative), particularly against Pseudomonas spp. Most isolates grew best at a temperature of 25 to 30 degrees Celsius. These results validated KSA 09's antagonistic action against phytopathogens, which is highly recommended.

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