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Characterization of antimicrobial potential of thermophilic actinomycetes isolated from different industrial sites

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ABSTRACT

Actinomycetes are unique, mostly thermophilic, filamentous, branched and conidia forming group of microorganisms widely distributed in nature, inhabiting diverse ecological niches such as soil, plant tissues, compost and aquatic environments. This study is aimed to isolate, characterize and identify Actinomycetes from soil samples exhibiting antimicrobial activity against 12 specific antibiotics. Soil samples were collected from various industrial sites in Durg district, including ACC Cement Jamul, Shyam Steel Industries, Kushal Chemicals, Techno Enterprises and Coal Chem Industries. A total of 35 isolates were obtained. The colonies were cultured on Actinomycete Isolation Agar (AIA) medium to produce pure cultures and preserved on slants. Five isolates (ACC-I, SH-I, KC-I, TC-I, and CC-I) were screened, purified and analyzed on the basis of morphological and cultural characteristics. Gram staining was performed on selected isolates and all Gram-positive isolates were tested primarily for their antibiotic sensitivity. The results revealed that all isolates exhibited substantial inhibition zones against Kanamycin, Gentamicin, Tetracycline, Sulfonamides, Rifampicin, Cephalosporins and Violamycin, whereas no activity was observed against Amoxicillin, Ampicillin and Penicillin. Notably, isolate SH-I displayed the largest inhibition zone against Azithromycin and ACC-I displayed smallest inhibition zone against sulfonamide and cephalosporin. This study highlights the potential of Actinomycetes from industrial soil as a source of novel antimicrobial compounds. These isolates demonstrated antibacterial activity and may be utilized in the development of new antibiotics for pharmaceutical or agricultural applications.

Keywords: - Actinomycetes, Antimicrobial, Antibiotic sensitivity, Industrial soil, Inhibition zone

INTRODUCTION

Soil microorganisms are an important source for discovering and identifying Actinomycetes with therapeutic potential. Among them, Actinomycetales is an important order (Khanna *et al.,* 2011). The order Actinomycetales is composed of about 80 genera, nearly all from terrestrial soils, where they live primarily as saprophytes, in water, and colonizing plants, showing marked chemical and morphological diversity but belonging to a distinct evolutionary line (Barka *et al.,* 2016). Thermophilic Actinomycetes are thriving at relatively high temperatures, typically between 40 - 80°C (Kanekar and Kanekar, 2022). Actinomycetes are Gram positive bacteria with a high guanine cytosine content in their DNA (over 55%), renowned for their ability to produce diverse secondary metabolites, such as antibiotics and other biologically active substances with therapeutic potential (Chandurkar, 2022). Actinomycetes exhibit a filamentous growth habit with branching hyphae and conidia formation characteristics that closely resemble those of fungi. For this reason, they are also known as ray fungi (Anilkumar *et al.,* 2017). Industrial soil has been found to exhibit thermophilic Actinomycetes (Harwani, 2013).Microorganisms in the soil increase the availability of elements like phosphorus, potassium etc. (Shakya *et al.,* 2025). Soil is a heterogeneous mixture of plant residues, animal remains and microbial biomass including Actinomycetes, which play a vital role in decomposing organic matter (Rathore *et al.,* 2015).

Actinomycetes produce aerial and substrate mycelium. *Streptomyces* is a dominant genus of Actinomycetes. A wide variety of Actinomycetes have the potential to produce numerous bioactive compounds with diverse clinical effects and significant applications in human medicine. It has been estimated that approximately one-third of the thousand of naturally occurring antibiotics have been obtained from Actinomycetes (De Simeis and Serra, 2021). The resistance problems challenge

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the discovery of new antibacterial agents effective against resistant pathogenic bacteria and fungi (Subramani and Aalbersberg, 2013). Therefore, it is essential to screen Actinomycetes from different habitats for antimicrobial activity with the possibility of discovering new Actinomycetes strains that produce antibiotics that have not yet been identified and are active against drug resistant pathogens.

MATERIALS AND METHODS

Soil Sampling and Pretreatment

Soil samples were collected from five industrial areas in the Durg district, viz., ACC Cement Jamul, Shyam Steel Industries, Kushal Chemicals, Techno Enterprises, and Coal Chem Industries for the purpose of screening Actinomycetes. Sampling involved removing the top layer of soil and collecting 4–5 cm of surface material using a spatula. The central portion of the soil was then collected with a trowel and stored in sterile plastic bags. The soil samples were incubated at room temperature for 24 hours before being stored at 4 °C for further processing.

Isolation of pure culture

To isolate and screen pure cultures, 1g of each soil sample was suspended in 10ml of sterile water and mixed thoroughly. Stock solutions were prepared by transferring 1ml of the mixture into a fresh test tube containing 9ml of sterile water, resulting in a five-fold serial dilution. This process was repeated five times. 0.1ml from each dilution was placed on Actinomycete Isolation Agar (AIA) media using the spread plate technique and incubated at 37±2°C for 7 days. After incubation, culture plates were examined and colonies displaying Actinomycetes characteristics were selected and purified using the streak plate technique. Purified colonies were transferred onto fresh AIA plates and slants for long-term preservation at 4°C.

Morphological Characterization

The identification of the selected Actinomycetes was carried out through both macroscopic and microscopic examinations, following the methodology described by Sapkota *et al.,* 2020). Observations included a detailed analysis of colony morphology on AIA, focusing on characteristics such as size, shape, configuration, elevation, margin, pigmentation, the presence or absence of aerial and substrate mycelium and overall colony structure. Each isolate was streaked onto a plate and incubated at 37 ± 2 °C for seven days. During this incubation period, the morphological characteristics of the colonies were examined daily and carefully recorded to facilitate accurate identification of the isolates. These morphological features were documented as part of the identification process.

Primary Screening

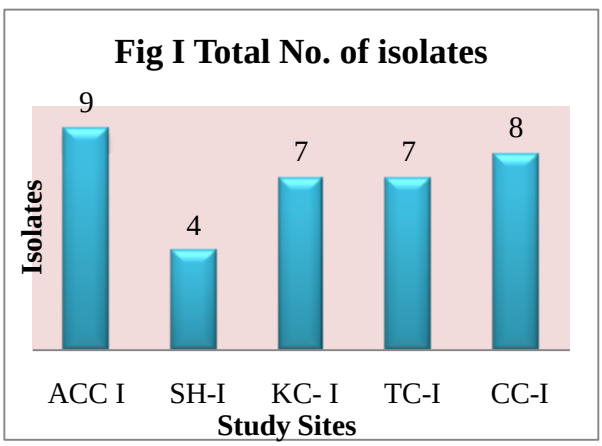
Gram staining was performed on isolates involving crystal violet staining, iodine solution treatment and decolorization with 95% ethanol, safranin, washing, and compound microscope examination. Antibiotic sensitivity tests were performed on isolates grown on AIA plates. In these tests, pure cultures of Actinomycetes were cultured using the spread plate method on Mueller Hinton Agar. Twelve different antibiotic discs viz. Amoxicillin, Rifampicin, Gentamicin, Cephalosporin, Penicillin, Ceftriaxone, Azithromycin, Sulfonamide, Tetracycline, Ampicillin, Violamycin and Kanamycin were placed on the freshly spread medium with care and incubated at 37±2°C for 24 hours to determine their growth. The zone of inhibition refers to the area around an antibiotic disc where Actinomycetes growth is inhibited. This zone indicates the effectiveness of the antibiotic against the isolate present on the agar plate. This analysis provides comprehensive insights into the properties of the isolates regarding their antibiotic susceptibility and resistance (Waksman *et al.,* 2010).

RESULTS

In this study, soil samples were collected from various industrial areas in Durg district. A total of 35 isolates were obtained, from which 5 isolates were selected for further investigation based on their morphological characteristics. The radial colony sizes of these isolates were measured, ranging from 0.1 to 1.0 cm. (Table 1 and Fig I). The isolates exhibited diverse pigmentation, with colony colors ranging from bluish, creamy, blackish, to white. This pigmentation is illustrated by the production of pigments by ACC-I, SH-I, KC-I, TC-I, and CC-I

Table 1: Isolation of Actinomycetes from different Industrial area

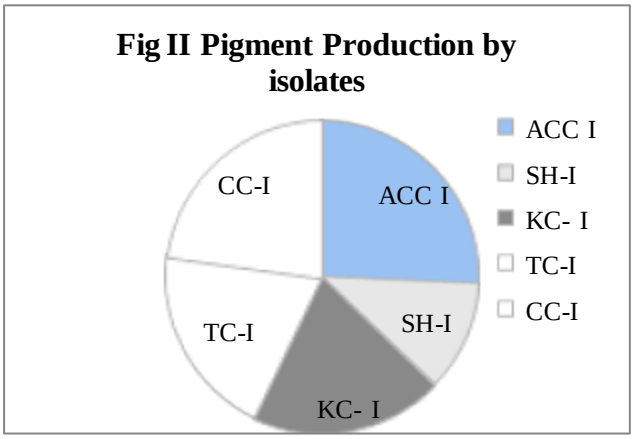
S. No.	Soil Sample	Industries	Total isolates	Selected Isolate	Radial Colony size (cm) after 7 days	Number of colonies
1.	S.S. I	ACC Cement Industries	09	Isolate –I	0.7	3
				Isolate -II	0.2	5
				Isolate -III	0.3	9
				Isolate -IV	0.8	5
				Isolate -V	0.2	4
				Isolate -VI	0.2	15
				Isolate –VII (ACC-I)	1.0	4
				Isolate - VII	0.1	1
				Isolate - VIII	0.1	2
2.	S.S. II	Shyam Steel Industries	04	Isolate -I	0.1	3
				Isolate –II (SH-I)	0.2	9
				Isolate –III	0.1	6
				Isolate –IV	0.1	9
3.	S.S. III	Kushal Chemicals	07	Isolate –I	0.1	6
				Isolate –II	0.2	7
				Isolate –III	0.3	8
				Isolate –IV	0.1	23
				Isolate –V	0.2	1
				Isolate –VI (KC-I)	0.4	5
				Isolate –VII	0.2	6
4.	S.S. IV	Techno Enterprises	07	Isolate –I	0.2	6
				Isolate –II	0.2	2
				Isolate –III	0.1	8
				Isolate –IV	0.1	10
				Isolate –V	0.3	5
				Isolate –VI (TC-I)	0.5	2
				Isolate -VII	0.1	15
5.	S.S. V	Coal Chem. Industry	08	Isolate –I	0.2	5
				Isolate –II (CC-I)	0.6	10
				Isolate -III	0.2	13
				Isolate -IV	0.2	9
				Isolate -V	0.1	8
				Isolate -VI	0.2	7
				Isolate -VII	0.3	6
				Isolate - VIII	0.2	17



isolates. Morphological characteristics, such as growth pattern, varied among the isolates. KC-I, TC-I, and CC-I exhibited filamentous and ACC-I and SH-I displayed circular and irregular growth pattern respectively. The elevation of the isolates also showed variation with ACC-I, SH-I, KC-I, TC-I, and CC-I exhibiting flat, raised, ovate, umbonate, and convex elevations, respectively. Furthermore the isolates displayed distinct margins SH-I and CC-I exhibited serrate margins, while ACC-I and CC-I had smooth margins. In contrast, KC-I displayed hairy margins (Table 2 and Fig II).

Table 2: Growth characteristics of isolates

Selected Isolate	Colony Size(cm)	Color (Pigmentation) of Colony	Growth Pattern	Elevation	Margin	Gram Staining (+/-)
ACC-I	1.0	Bluish	Circular	Flat	Smooth	+Ve
SH-I	0.2	Creamy	Irregular	Raised	Serrate	+Ve
KC-I	0.4	Blackish	Filamentous	Ovate	Hairy	+Ve
TC-I	0.5	White	Filamentous	Umbonate	Serrate	+Ve
CC-I	0.6	White	Filamentous	Convex	smooth	+Ve



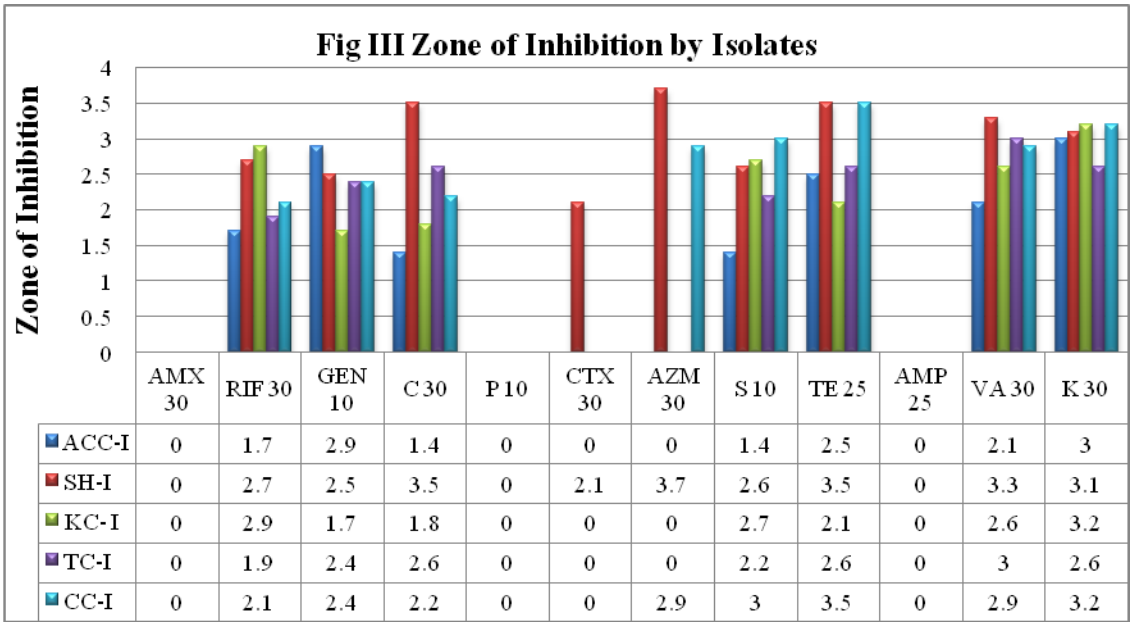
The cultural characteristics were studied in terms of size, pigmentation, growth pattern elevation, margin of the colonies and Gram staining of the isolates. The results revealed that all five isolates (ACC-I, SH-I, KC-I, TC-I, and CC-I) were gram-positive (Table 2). For primary screening of the potential antibiotic production, antibiotic sensitivity tests were performed against the selected isolates. The results showed that Kanamycin, Gentamycin, Tetracycline, Sulphonamide, Rifampicin, Cephalosporines, and Violamycine exhibited zones of inhibition in all five isolates. In contrast, Amoxicillin, Ampicillin, and Penicillin did not show any zone of inhibition in any of the selected isolates. Notably, Azithromycin and Ceftriaxone showed zones of inhibition only in isolate SH-I, with zones ranging from 2.1 to 3.7 cm. The largest zone of inhibition (3.7 cm) was observed in SH-I against Azithromycin and ACC-I displayed smallest inhibition zone against sulfonamide and cephalosporin (1.4 cm) (Table 3, Fig III and Fig IV)

Table 3: Antibiotic sensitivity test showing zone of inhibition

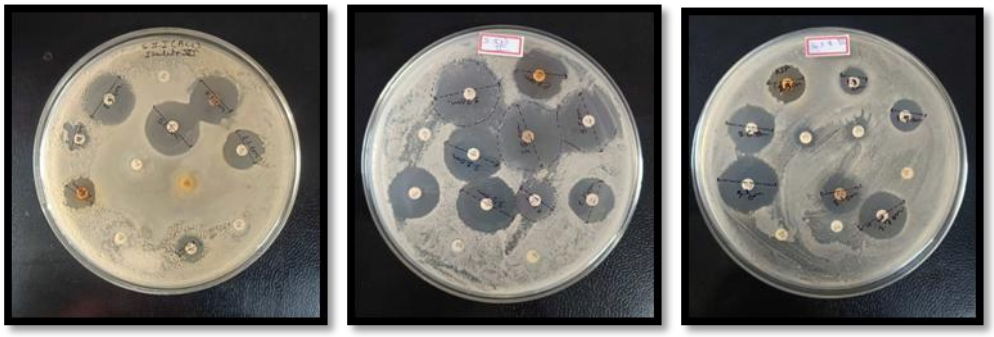
Antimicrobial agent	Zone of Inhibition (cm)				
	(ACC-I)	(SH-I)	(KC-I)	(TC-I)	(CC-I)
Amoxicillin (AMX 30)	Nil	Nil	Nil	Nil	Nil
Rifampicin (RIF30)	1.7	2.7	2.9	1.9	2.1
Gentamicin (GEN10)	2.9	2.5	1.7	2.4	2.4
Cephalosporin (C 30)	1.4	3.5	1.8	2.6	2.2
Penicillin (P10)	Nil	Nil	Nil	Nil	Nil
Ceftriaxone (CTX30)	Nil	2.1	Nil	Nil	Nil
Azithromycin (AZM30)	Nil	3.7	Nil	Nil	2.9
Sulfonamide (S10)	1.4	2.6	2.7	2.2	3.0
Tetracyclines (TE25)	2.5	3.5	2.1	2.6	3.5
Ampicillin (AMP25)	Nil	Nil	Nil	Nil	Nil
Violamycin (VA30)	2.1	3.3	2.6	3.0	2.9
Kanamycin (K30)	3.0	3.1	3.2	2.6	3.2

DISCUSSION

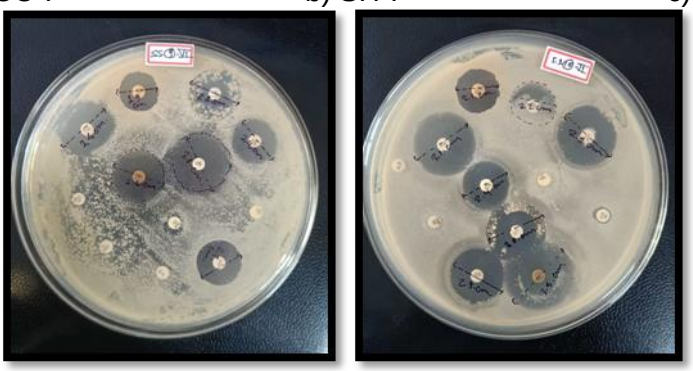
The present study provides valuable insight into the potential of antimicrobial producing Actinomycetes isolated from the industrial site. The isolation of five Actinomycetes spp. from industrial soil samples highlights the resilience of these microorganisms in adapting to challenging environments. The unique morphological characteristics of these Actinomycetes, including filamentous and circular structures, as well as their ability to produce diverse colored aerial and substrate mycelia, underscore their ecological significance. The majority of these organisms produced pigments, which may play a crucial role in their



	AMX 30	RIF 30	GEN 10	C 30	P 10	CTX 30	AZM 30	S 10	TE 25	AMP 25	VA 30	K 30
ACC-I	0	1.7	2.9	1.4	0	0	0	1.4	2.5	0	2.1	3
SH-I	0	2.7	2.5	3.5	0	2.1	3.7	2.6	3.5	0	3.3	3.1
KC-I	0	2.9	1.7	1.8	0	0	0	2.7	2.1	0	2.6	3.2
TC-I	0	1.9	2.4	2.6	0	0	0	2.2	2.6	0	3	2.6
CC-I	0	2.1	2.4	2.2	0	0	2.9	3	3.5	0	2.9	3.2



a) ACC-I b) SH-I c) KC-I



d) TC-I e) CC-I

Fig IV: Zone of Inhibition showing Antibiotic Sensitivity (a-e)

adaptation to industrial pollution. The ability of these Actinomycetes to thrive in polluted soils and enhance their populations in response to industrial effluents suggests their potential applications in bioremediation and agricultural practices. Moreover, their capacity to produce bioactive compounds positions them as valuable resources for developing new antibiotics and other therapeutic agents**.** The role of these microorganisms in promoting plant growth and protecting against phytopathogens further emphasize their importance in sustainable agriculture and their significant benefit to society (Koskey *et al.,* 2021). Soils possess significant potential as sources of new antibacterial compounds active against pathogenic microorganisms due to their rich floral and microbial biodiversity, which provides a

promising habitat for Actinomycetes (Chaudhary *et al.,* 2013). An attempt in this piece of research has been made to isolate the thermophilic Actinomycetes from unexplored regions in order to find novel species and their antibiotic producing efficiency.

CONCLUSION

This study led to the successful identification of five Actinomycetes from soil samples collectedfrom the Industrial area, all of which exhibited significant antimicrobial activity. Notably, all isolated strains demonstrated substantial antibiotic activity with a remarkable inhibition zone formed against Kanamycin. Conversely, no zone formation was observed against Amoxicillin and Ampicillin highlighting the specificity of their antimicrobial properties. These findings underscore the pressing need for the discovery of novel antimicrobial producing strains. The alarming rise in antimicrobial resistance among microorganisms has rendered existing drugs increasingly ineffective, posing a significant threat to global health. The identification of new antimicrobial agents is crucial to addressing this challenge and developing innovative therapeutic strategies to combat infectious diseases. The results of this study contribute to the ongoing search for novel antimicrobial compounds and highlight the potential of Actinomycetes species as a rich source of new antibiotics.

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