Isolation and Characterization of *Actinomycetes* from Different Soil Samples (Ghaziabad) & Screening their Antibacterial Activity

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ABSTRACT

The name actinomycetes is derived from the Greek words akitis (lightning) and mykes (mushroom or fungus), and is used to refer to fungi as prokaryotic microorganisms. They are very diverse and can be found in most natural environments. These are prokaryotes with extremely diverse metabolisms. The ability and production of a range of essential health substances, such as antibiotics, enzymes, and immunomodulators; they are common in soil, water, and planting plants. Because a person suffers from a disease caused by infectious microorganisms, he begins to look for a treatment, which leads to the discovery of a large number of antibiotics in the microorganisms. Microbes have made outstanding contributions to the health and well-being of people all over the world. They are the source of many commercial antibiotics. Actinomycetes are an important class of gram-positive filamentous bacteria that can produce antibiotics for agricultural and medical purposes. In this study, soil samples were taken from the Ghaziabad region. A total of 9 strains of actinomycetes were isolated from Ghaziabad. These Actinomycetes were screened with respect to potential against Gram-positive and Gram – negative bacteria. Soil sample was screened for their antibacterial activity. They were examined for their inhibitory activities in 3 test organism. The cultural properties of the isolates were also examined in various culture media. The results indicated that 9 *Actinomycetes* isolates were highly active against *Bacillus, Staphylococcus aureus* and *E.coli* strains. 9 *Actinomycetes* isolates were highly active with an inhibition zone more than 15 mm in diameter. All the antibiotic producing Actinomycetes were isolated at 28°C from soil. 9 *Actinomycetes* isolates showed activity against bacteria in which most of them from alkaline soil.

Keywords: Prokaryotic microorganisms, *Bacillus, Staphylococcus aureus, E.coli*, Filamentous bacteria, Immunomodulators

Introduction

Infectious diseases caused by resistant bacteria and fungi are a serious global problem [1]. To combat them, new antimicrobial agents must be found. actinomycetes are an important source of biologically active substances with important medical and economic significance. Especially in the field of biotechnology [2]. Approximately two-thirds of antibiotics come from actinomycetes [3], most of which are produced by different *Streptomyces* species. This group of bacteria is interesting because it has a complex life cycle and its members produce various types of antibiotics [4-6]. *Streptomyces* examples include the antibacterial agents chloramphenicol, clindamycin, erythromycin, imipenem, streptomycin, tetracycline, and the antifungal agents amphotericin B and nystatin. Other biologically active compounds include anti-cancer compounds, such as echinosporine [7] and Limocrocin, an immunosuppressant, rapamycin, a macrolide insecticidal compound (Avermectin) and herbicide (Glufosinate), also produced by Streptomyces spp. Ruan (1994) reported about 1000 species of rare actinomycetes, including 400 species of *Micromonospora*, 270 species of Nocardia, 170 species of actinomycetes, 150 species of actinomycetes, 50 species of *Saccharopolyspora* and 40 species of Spore fungus. Such as rifamycin produced by *Amycolatopsis mediterranei*, erythromycin produced by *Saccharopolyspora erythraea*, teicoplanin produced by

Teichomyceticus, gentamicin and vancomycin produced by *Amycolatopsis orientalis and Micromonospora purpurea*. In the past 20 years, the number of discoveries of new antibiotics from various sources has declined, while the demand for antibiotics for the treatment of drug-resistant pathogens and opportunistic diseases has steadily increased by AIDS patients and organ transplant patients. Therefore, it is important and necessary to find the source of new antibiotics [8], and actinomycetes may still be the most interesting source.

Actinomycetes are the most abundant species of microorganisms in nature. They constitute an important part of the soil microbial population [9]. *Streptomyces* is the dominant genus among actinomycetes. About 90% of actinomycetes isolated from soil belong to the genus *Streptomyces* [10]. Obviously, some actinomycetes, especially *Streptomyces* and filamentous fungi, and to a lesser extent several bacterial species, are the main producers, both in terms of the number, versatility and structural diversity of the metabolites produced aspect. In the past few decades, the importance and frequency of these important microorganisms as producers of biologically active metabolites has changed significantly; at the beginning of the antibiotic era, fungal-derived antibiotics (penicillin, griseofulvin) and bacteria (Gramicidin) is in the foreground. Nowadays. Interestingly, after the discovery of streptomycin, then chloramphenicol, tetracycline, and macrolides, people began to pay attention to *Streptomyces* species. In the 1950s and 1960s, most (70%) antibiotics came from these types. Over the next 20 years, the importance of species other than *Streptomyces* actinomycetales (rare actin) has increased, producing 25-30% of all known antibiotics.

Soil is a complex and very diverse environment that provides a versatile source of antibiotic-producing organisms [11]. Around 500 antibiotics were found annually, 60% of which are obtained from the soil [12]. Recent analysis has shown that soil screening for antibacterial activity has been carried out in many parts of the world [13]. *Streptomyces* is responsible for the production of more than 60% of the known antibiotics, and the other 15% are produced by various *Actinomycetes, Micromonospora, Actinomadura, Streptoverticillium* and *Thermoactinomycetes* [14]. Actinomycetes account for about 70% of them, and the remaining 30% are the products of filamentous fungi and non-actinomycete bacteria [15].

According to the World Health Organization over prescription and the improper use of antibiotics has led to the generation of antibiotic resistance in many bacterial pathogens [9]. *Staphylococcus aureus, E.coli,* and *Bacillus* strains are virulent pathogen that is responsible for a wide range of infections and has developed resistance of most classes of antibiotics. Hence there is need to rediscover new drugs active against these drugs resistance pathogens. Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi. This study aims to isolate actinomycetes from soil samples in Ghaziabad and evaluate their antibacterial properties. The problem of drug resistance requires the discovery of new antibacterial agents that are effective against pathogens resistant to modern antibiotics. Therefore, it is necessary to analyse more and more actinomycetes with antibacterial activity from different habitats to obtain some actinomycete strains that produce undetected antibiotics and are effective against drug-resistant pathogens. These organisms are responsible for the characteristic musty or earthy smell of a freshly plowed field due to the volatile matter they produce.

Method and Materials

1. Sample collection

The soil samples were collected from various areas of Ghaziabad. Approximately 250gm of soil sample was collected for further processing. Soil sample was collected in such way to get the soil of crust and depth of a minimum of 10cm with the assistance of sterile spatula and placed in sterile plastic bags for transportation to laboratory. These were air dried for 1 week and crushed and sieved. The sieved soil was used for Actinomycetes isolation.

2. Pre-treatment

All soil Sample had been mixed with calcium carbonate & pretreated for 2-5days at 37°C. 1gm soil mixed with 0.1g Calcium carbonate & incubates at 37°C for 2-5 days. This pretreatment enhances the population of *Streptomycetes spp*. in soil samples.

3. Isolation of Actinomycetes

Isolation and enumeration of Actinomycetes were done by serial dilution and spread plate technique. One gram of soil was suspended in 9ml of sterile double distilled water and then the dilution was carried out up to 10^{-5} dilutions. Aliquots [0.1ml] of 10^{-2} ,$10^{-3,}$ 10^{-4} and 10^{-5} were spread on the Actinomycetes isolation agar. Plates were then incubated at room temperature (28 to 30 °C) for 24 hours. The plates were observed intermittently for the Actinomycetes growth during incubation. After incubation, Actinomycetes colonies which are morphologically distinct were picked from the Actinomycetes isolation agar plates and further purified by repeated streak plate method. Once the pure colonies were obtained, each colony was further identified based on its characteristics such as colony, morphology, the color of hyphae and the presence or absence of aerial and substrate mycelium. Then, selected and identified colonies of Actinomycetes were transferred to starch casein agar slant and incubated at 27 °C for their growth. After incubation, the slants containing pure *Actinomycetes* isolates were stored at-4 °C for further studies.

Test Organisms- Antibacterial activities were performed against the *Bacillus, Staphylococcus aureus* and *E.coli* etc.

4. Characterization and identification of Actinomycetes

Characterization of potent actinomycete isolates was carried out by morphological, biochemical and physiological studies as described in the *International Streptomyces Project* [ISP] [16].

I. Morphological Identification

Gram staining and lacto phenol blue staining were done to study the morphology of the Actinomycetes cells and spore chain morphology was studied coverslip culture technique with a light microscope.

Gram Staining

A smear of culture was taken in a clean glass slide and heated gently over a flame. The smear was covered with a thin film of crystal violet for 1 minute and washed gently in slow running tap water. Gram's iodine solution was flooded over the smear for 2 minute and washed with tap water. Alcohol was used to decolorize the smear until the violet color ceased to flow away**.** Then the slide was washed with water and counter stain safranin was flooded over the smear for 2 minutes then the slide was washed, drained, air, dried and viewed under microscope. The culture retaining the violet color indicated that it was Gram positive organisms.

II. Physiological and cultural characterization

Colony morphology of the isolates of actinomycetes was studied under a high-power magnifying lens by observing color of the colony, nature of the mycelium, spore surface and felling the consistency with a sterile loop.

III. Biochemical characterization

The isolate was biochemically characterized according to the Berge's Manual of Determinative Bacteriology including Catalase test, nitrate reduction test, IMVIC test, Starch hydrolysis test, Fermentation of citrate test, Triple sugar iron test, Citrate utilization test, Skim milk agar hydrolysis, Hydrogen sulfide test performed to check the biochemical characteristics of producing strain.

5. Screening of Isolates for Antibacterial activity

The actinomycete isolates are selected to demonstrate the antibacterial activity against the pathogen test organism by diffusion in the agar wells in the agar medium. The Actinomycetes isolates usually encounter show antibacterial activity on potato dextrose agar media. Most active isolates are active against Gram-positive and Gram-negative pathogens. Test the antibacterial activity against *Bacillus, Staphylococcus aureus* and *Escherichia coli* by diffusion in agar wells.

Results and Discussion

Soil samples were collected from waste land soil from Ghaziabad. 1 gram of soil sample was dried and taken for isolation of Actinomycetes. The 9 Actinomycetes were isolated from 20 Soil Samples at two different temperatures 28°C or 37°C [Table 1]. All the cultures were screened against bacteria but only the 9 isolates

showed the antibacterial activity and were designated as A1, A2, A3, A4, A5, A6, A7, A8, A9 [Table 2]. They were also studied for culture characteristics.

This study was undertaken with an aim of isolation and screening of Actinomycetes in soil from Ghaziabad region and selective media and cultivation conditions described previously a total of 9 different Actinomycetes isolates were recovered from 20 soil samples that were collected from Ghaziabad. The soil sample from Modinagar and Raj Nagar in Ghaziabad gives the higher number of Actinomycetes isolates [Table 1].

All isolates grow on isolation agar media showing morphology typical of Actinomycetes. Since the colonies were slow growing, aerobic, folded and with aerial and substrate mycelia of different colors. All actinomycetes isolates were Gram's stain positive. The cultural characteristics (pigment production), morphological characteristics of the different actinomycetes isolates are presented in [Table 2].

Out of 9 Actinomycetes subjected for primary screening and subjected for purification methods by streak plate method. The Identification of the potent antibiotic producing strains reveals that all the strains belong to the genus *Streptomyces.* The isolated microorganism was Gram positive, having branching and were filamentous. Different isolates showed varying results in the Biochemical test as shown in [Table 3].

Out of 20 isolates the 9 isolates were showed positive antibacterial results. These isolates were selected for their broad spectrum of activity and zone of inhibition in mm.

Table 1. Total number of Actinomycetes Isolates with Antibacterial activity isolated at different temperature.

Origin	Isolation temperature	Total strains isolated	No. of active Isolates against bacteria
Waste land soil near Muradnagar	28°C, 37°C	1	1
Garden soil near Modinagar	28°C, 37°C	8	3
Garden soil near Raj Nagar	28°C, 37°C	9	4
Waste land soil near Mohan Nagar	28°C, 37°C	2	1
Total		20	9

Table 2. Culture Characteristic of Selective isolates on Isolation agar medium.

Origin	Culture Code	Color	Mycelium type	Pigment production	Gram's reaction
Waste land soil near Muradnagar	A1	Green	Aerial	Black	+
Garden soil near Modinagar	A2	White	Aerial	Orange	+
	A3	Dark green	Aerial	Black	+
	A4	White	Substrate	Yellow	+
Garden soil near Raj Nagar	A5	White	Aerial	Orange	+
	A6	Green	Aerial	Yellow	+
	A7	White	Aerial	Orange	+
	A8	White	Aerial	Yellow	+
Waste land soil near Mohan Nagar	A9	White	Aerial	Orange	+

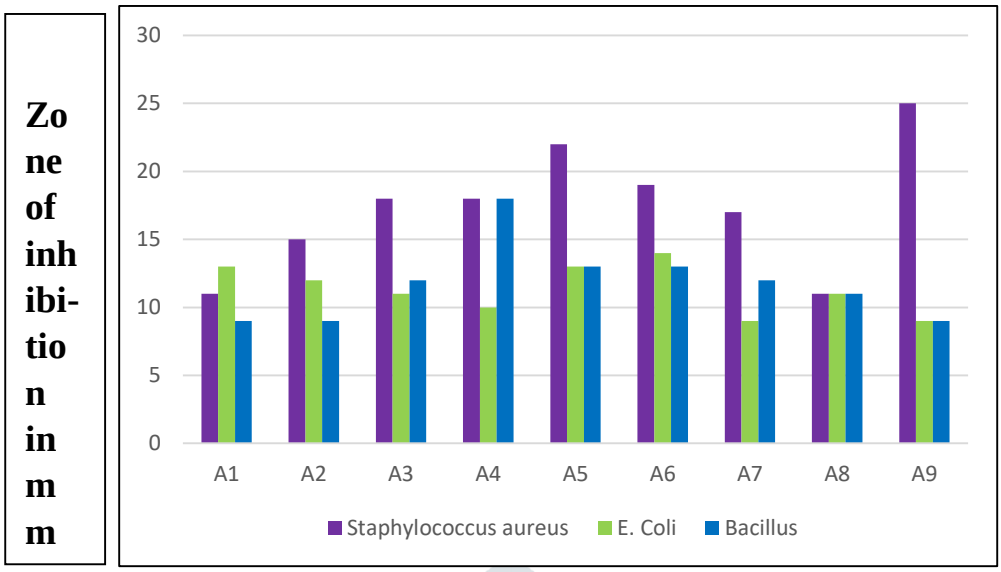
Table 3. Biochemical characterization of *Actinomycetes* isolates

Biochemical tests	A1	A2	A3	A4	A5	A6	A7	A8	A9
Nitrate reduction	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-
MR	-	-	-	-	-	-	+	+	+
VP	-	-	-	-	-	-	-	-	-
Citrate	+	-	+	+	-	-	+	-	-
TSI (Slant)	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	-	+	-	+	+	-	+	+
Skim milk agar hydrolysis	-	-	-	-	-	-	-	-	-
Hydrogen sulfide production	-	-	-	-	-	-	+	-	-
Catalase test	+	+	+	+	+	+	+	+	+

Table 4. Antibacterial activity of isolates with zone of inhibition in mm (Agar well diffusion methods).

Culture Code	*E. Coli*	*Bacillus*	*Staphylococcus sp.*
Concentration Of antibiotic	**25%**	**25%**	**25%**
A1	13	9	11
A2	12	9	15
A3	11	12	18
A4	10	18	18
A5	13	13	22
A6	14	13	19
A7	9	12	17
A8	11	11	11
A9	9	9	25

Graphical representation of Antibacterial activity of isolated strains



Conclusion

Nine isolates showed antibacterial activity, mainly from Ghaziabad. These microorganisms produce some of the most important drugs ever and are an important source of treatment for bacterial and fungal infections. The number of terrestrial antibiotics is currently approaching the saturation curve, and there will be obvious limits in the near future. More and more leading pharmacological antibiotics are used for new treatments of drug-resistant infectious pathogens, which has intensified the search for metabolites in primitive habitats with much less human activity. Our research will enable us to establish abundant and diverse actinomycetes in the region, especially in the various niches of Ghaziabad, and help protect and utilize them in the bio industry.

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