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Biodiversity of *Actinomycetes* Species Isolated from Saline Belt of Akola District

KEYWORDS: Actinomycetes species, Antibiotic producer, Antagonistic activity.

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ABSTRACT *Actinomycetes are best known for their ability to produce antibiotics and are Gram positive bacteria which are group of branching unicellular organism. The present study was designed to isolate and identify the different actinomycetes species from saline soil and to evaluate their antimicrobial activity against multidrug resistant bacteria. Total fifteen isolates such as Streptomyces sp., Nocardia sp., Streptomyces sp. shows higher antibacterial activity against Gram +ve bacteria whereas Nocardia species shows activity against Gm –ve bacteria. The antagonistic activity was studied using the agar well diffusion method against the MDR clinical pathogens thus, the results of present investigation revealed that soil Actinomycetes are the potent source of novel antibiotics and were found to be of potential antagonistic activity against test organisms which can control variety of pathogenic organisms.*

INTRODUCTION

Actinomycetes are of universal occurrence in nature and are widely distributed in natural and manmade environments. They are found in large numbers in soils, fresh waters, lake (Bizuye et al., 2013). Actinomycetes was derived from Greek "atkis" (a ray) and 'mykes" (fungus) and has features of both bacteria with fungi (Das et al., 2008) . Actinomycetes are filamentous, branching bacteria with fungal type of morphology. They are part of the microbial flora of most natural substrates. (Moustafa et.al., 1962)

Actinomycetes are gram positive bacteria frequently filamentous and sporulating with DNA rich in G=C From 57-75% (Lo et al., 2002). Actinomycetes are originally considered as an intermediate group between bacteria and fungi (Mohan Remya and Ramasamy Vijaykumar, 2008).

Actinomycetes are classified as heterotropic prokaryotes and referred as filamentous bacteria with different morpholiogical, cultural and biochemical characteristics. They constitute a large portion among the rhizospheric microbiota.

The role of Actinomycetes in organic waste degradation has been documented due to its ability to secret extracellular enzymes viz. chitinase, ligninase, xylans and pectinase.

Actinomycetes are a potential source of novel compounds as the environmental conditions of the sea are entirely different from the terrestrial conditions (Meiying and Zhicheng 1998) They play a major role in recycling of organic matter production of novel pharmaceuticals, nutritional material, cosmetics, enzymes, antitumour agents, enzyme inhibitors, immune- modifiers and vitamins. (Mohan Remya and Ramasamy Vijaykumar, 2008) Secondary metabolites are produced by some organisms such as bacteria, fungi, plants, Actinomycetes and so forth. (Abebe Bizuye et al., 2013). Actinomycetes are known for causing diseases in humus, and for the important role they play in soil ecology. They produce a numbers of enzymes that help to degrade organic plant materials lignin, and chitin. It is especially significant for their role on the recycling of organic matter (Shrinivasan et al., 1991). Actinomycetes with the potential to yield new products. Need of new antimicrobial agents is greater than ever because of emergence of multidrug resistance in common pathogens, the rapid emergence of new infections and the use of multidrug resistance pathogens in bioterrorism (Spelberg et al., 2004).

Antibiotics has been used in many fields including agriculture , veterinary and pharmaceuticals Industry, Actinomycetes have the capability to synthesize many different biologically active secondary metabollities such as antibiotics, herbicides, pesticides, anti-parasitic and enzyme. Almost 80% of the world's antibiotics are known to come from Actinomycetes, mostly Streptomyces and Micromonospora (Pandey et al., 2004). Present time most of diseases caused by bacteria have become resistant to most of time the antibiotics (Alanis, 2005).

The range of the antimicrobial activity of Actinomycetes can be tested by two methods, base on the ability of antibiotics produced on the Actinomycetes to differ into agar and either retard the growth of microorganism within the diffusion zone of the active substance or inhibit it completely.

Hence the present investigation entitled "Biodiversity of Actinomycetes Species Isolated from Saline Belt of Akola District" have been undertaken to study the diversity of Actinomycetes from saline soil and to study their antagonistic activity.

MATERIAL AND METHODS

The present work has been undertaken to study the "Biodiversity of Actinomycetes species isolated from Saline Belt of Akola District". The experiments were carried out adopting the following material and methods.

Collection of soil samples:

- The soil samples were collected from saline belt in and around Akola.
- Soil sample (Approx. 500 g) were collected using clean, dry and sterile polythene bags along with sterile spatula, marking pen rubber band and other accessories.
- Samples are dried separately at 45 °C for 1 hour in a hot air oven and then cooled to room temperature.
- Each of the soil sample collected (1g) was taken in a conical flask containing 100 ml of sterile water and few drops of Tween 80 was added.
- The flasks were shaken for 30 mins in an Orbital Shaker Incubator at 27 °C and their contents are designated as stock cultures (Casida A. L.1984).

Isolation and Identification of Actinomycetes from Saline soil

- Isolation of Actinomycetes was performed by serial dilution and plating technique using Starch Casein Agar medium.
- One gram of this soil sample was suspended in 25 ml

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sterile water in a conical flask, stirred thoroughly with the help of a glass rod and left for some time.
- Distilled water (9ml) was taken in each of the 7 test tubes and labeled from 1 to 7. The supernatant liquid from the dissolved soil sample was transferred into the test tubes so as to achieve the serial dilutions of 10^{-1} to 10^{-7}.
- 1 ml of the diluted sample was inoculated in the Starch Casein Agar medium and Actinomycetes Isolation Agar plates from each dilution. The Petri plates are then rotated to spread the sample uniformly. Plates were then incubated at room temperature (28 to 30ºC) for 84hrs (Hayakawa M. et al., 1995; Tamura T. et al., 1991; Augustine S. K. et al., 2004).
- After 72 hrs, white pin-point colonies, characteristic of Actinomycetes with a clear zone of inhibition around them were seen. The pinpoint colonies with inhibitory zone of inhibition were selected and purified into Actinomycetes Agar slants.
- The selected strains were further purified by multiple streaking methods. The stock cultures of each selected strain was prepared and maintained in Actinomycetes agar slants at +4°C.
- The Actinomycetes colonies isolated from the crowded plate were selected for the further studies and labelled as strain 1, strain 2, strain 3.

Antagonistic activity of Actinomycetes species:
- Actinomycetes isolates were grown in 50 ml of Starch Casein Broth by submerged culture containing in 250 ml flasks by incubating at 32ºC for 7 days and centrifuged at 10,000 rpm for 15 min and the clear supernatant broth samples were tested for their antagonistic activity against the selected pathogens by agar well diffusion method (Saadoun I. and A. Mahana, 2008).
- Wells of 6mm diameter were prepared in the Nutrient agar plates (Mitra A.et al., 2008).
- The wells were filled with the 50µl of culture supernatant and the diameter of inhibition zones were measured after incubation for 24 hr at 37ºC.

RESULTS AND DISCUSSION

Observation Table:-

Table 1 : Morphological characteristics of Actinomycetes species

Isolates	Mycelium and nature of colony	Colour of mycelium	Gram stain
S1	Extensively branched, floccae Aerial substrate Mycelium	Ash	+
S2	Smooth, granular, Aerial & substratre Mycelium	White pink	+
S3	Smooth, hairy, raised wrinkled Aerial mycelium	White	+
S4	Mycellium	White	+
S5	Hook like Aerial mycelium	Brown	+
S6	Smooth hairy	Pink	+
S7	Raised wrinkled	Greyish White	+
S8	Aerial mycelium	Brown	+
S9	Hairy, raised	Grey	+
S10	Wrinkled Aerial mycelium	White	+
S11	Substrate mycelium	Yellow	+
S12	Spiral like mycelium	Ash	+
S13	Granular Aerial	Brown	+
S14	Raised, wrinkled mycelium	White	+
S15	Aerial substrate mycellium	Pink	+

+ = Positive, - = Negative

Table 2: Biochemical characters of Isolates

Test Performance	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
A. Fermentation of Sugars															
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. IMViC Test															
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C. Enzyme Study															
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatinase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ positive , - negative

Table 3: Antagonistic activity of Isolated strain of Actinomycetes sp. against clinical pathogens.

Isolates	Measurement of zone of inhibition in mm							
	S1	S2	S3	S4	S5	S6	S7	S8
S. aureus	24	26	17	22	23	25	28	30
E. coli	16	19	20	18	22	14	11	19
P. vulgaris	18	18	27	25	26	19	18	14
B. subtilis	29	23	21	22	21	11	14	12

Table 4: Antagonistic activity of Isolated strain of Streptomyces sp. against clinical pathogens.

Isolates	Measurement of zone of inhibition in mm			
	S9	S10	S11	S12
S. aureus	26	23	28	27
E. coli	18	20	17	20
P. vulgaris	13	17	16	18
B. subtilis	18	15	25	20

Table 5: Antagonistic activity of isolated strain of Nocardia sp. against clinical pathogens.

Isolates	Measurement of zone of inhibition in mm		
	S13	S14	S15
S. aureus	30	32	31
E. coli	19	16	19
P. vulgaris	19	17	15
B. subtilis	21	22	19

RESULTS AND DISCUSSION:-

A total of 15 Actinomycetes strains were isolated from hypersaline soil of Akola District and were further subjected to identification. Selective isolation of an organism was done on Actinomycetes Isolation Agar and Starch Casein Agar. The identified strain were Actinomycetes spp. (8), Streptomyces spp. (4) and Nocardia spp. (3) and were confirmed on the basis of Bergey's Manual of Determinative Bacteriology. All the isolates were found to be Gram positive and shows different colony colour on selective media. Similarly Hacene (1996, 1998, 2000) reported the screening and isolation of promising strains of Actinomycetes with potential antibiotics is a thrust area of search since many years.

The study of biochemical parameters showed that all the isolates obtained show Indol and Vogus Prauskar test negative

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where as Methyl red and citrate test positive. Most of the enzyme test was found to be positive.

With the increase in use of antibiotics, the serious problem arises resulting in the development of multidrug resistant bacteria. Actinomycetes are considered as the potent source of antibiotic producer. So, when these isolation were tested against the clinical pathogens all the isolates showed broad spectrum antimicriobial activity.

The antagonistic activity was studied using the Agar Well Diffusion method with the different clinical pathogens. Similar findings were reported by Usha Rakshanya (2011) as the Antagonistic activity of Actinomycetes isolates against human pathogens. All the isolates strains were seperated tested against the clinical pathogens such as S. aureus, E. coli, P. vulgaris, and B. subtilis. It was observed that after incubation period out of 15 isolated strains of Actinomycetes species strain number 4, 7 and 8 were significantly supress the growth of S. aureus. Overall the result revealed that the Actinomycetes strain from S1 to S8 shows strong antibacterial activity against the S. aureus.

By considering the other clinical pathogens the maximum zone of inhibition 29 mm was recorded in case of B. subtilis followed by 26 mm zone in case of P. vulgaris comparing all the results. It was observed that the isolates strains of Actinomycetes were having the strong antibacterial activity. The formation of inhibition zone around the pathogen strain is due to production of secondary metabolities by Actinomycetes species.

From the observation table it was observed that followed by Actinomycetes spp. The Streptromyces strains were also found to control the S. aureus organism. Similar findings were reported by Usha Rakshanya (2011) showed Streptomyces spp. exhibited high antibacterial activity against Staphylococcus aureus. The highest zone of inhibition 28 and 27 mm was recorded in case of S11 and S12 strain, followed by B. subtilis 25 and 20 mm respectively. Whereas, these strain shows moderate activity in controlling the other clinical pathogens. Similar results were showed by Bull et al., (2007), Jimenez-Esquilin et al., (2005) and reported that Streptomyces particularly is a profilic producer of secondary metabolities with several of its species have been isolated and screened from soil extensively.

The three different strain of Nocardia species was identified during the study. The highest zone of 32 mm against S. aureus organism was recorded followed by 31 mm in S15 and 30 mm in S13 strain. Overall results showed moderate activity in controlling the other pathogens.

Thus, the result of present investigation revealed that soil Actinomycetes are the potent source of novel antibiotics and were found to be of potential antagonistic against test organisms which can control variety of pathogenic organisms.

CONCLUSION

The present investigation entitled, "Biodiversity of Actinomycetes species isolated from saline belt of Akola District" have been studied and the following conclusion was drawn. Hypersaline soil is rich in Actinomycetes species, so we observed Biodiversity of Actinomycetes in saline belt of Akola district. All the isolated strains of Actinomycetes were able to control the clinical pathogens and hence they were able to produce antibiotic against Gram positive and Gram negative organisms.

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