

Original Research Article

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Screening and Isolation of Pigment Producers and Non-pigment Producers Actinomycetes from Rhizospheric Soil Sample

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ABSTRACT

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Actinomycetes are Gram negative and Gram positive filamentous and sporulating bacteria and represent a ubiquitous group of microbes widely distributed in natural ecosystem around the world. For the isolation of Actinomycetes various methods can be performed on the basis of different sources and media. In the present study, 8 Actinomycetes were isolates from rhizospheric soil from Karanja (Lad) District wahism. Out of 8 Actinomycetes isolates, two isolates was found to be sensitive against all isolates and shows very excellent zone of inhibition. Antagonistic activity against human pathogens like, *S. aureus*, *E. coli*, *P. aeruginosa* and *P. vulgaris*, was also carried out. From the complete observation it was noticed that isolate N obtained from neem soil and H from Hibiscus soil is having excellent antagonistic activity in controlling all the test pathogens.

Introduction

Rhizosphere is a unique biological niche with diverse microflora comprising bacteria, fungi, protozoa and algae. It is defined as the soil adjacent to an influenced by plant root exudates (Gonzales-France *et al.*, 2009). Actinomycetes form a large and important segment of the microflora of most natural environments. Soils, freshwater, lake and river bottoms, manures and compost contain an abundance of these organisms (Wright 1956a, Wright 1956b).

Actinomycetes are diverse group of Gram positive bacteria that usually grow by filament formation. They belong to the order Actinomycetales (Super kingdom: Bacteria,

Phylum: Firmicutes, Class: Actinobacteria, Subclass: Actinobacteridae) (Okami and Hotta 1988).

Actinomycetes species synthesize a numerous natural metabolites with diverse biological activity such as antibiotics. Antibiotics of Actinomycetes origin evidence a wide variety of chemical structure, including amino glycosides, antracyclines, β -lactams, nucleosides, peptides, polyenes, actinomycins and tetracycline (Barrios-Gonzalez *et al.*, 2005). Antibiotics have been used in many fields including agriculture, veterinary and pharmaceutical industry (Kavitha, Vijayalakshmi, 2007). Actinomycetes has

diverse clinical effects and are active against many pathogenic organisms Actinomycetes and their bioactive compound show antibacterial and anti microbial against various pathogens and multi drug resistant pathogens e.g. Vancomycin-Resistant *Enterococci*, *Methicillin-Resistant Staphylococcus aureus*, *Shigella dysenteriae*, *Klebsiella sp.* and *Pseudomonas aeruginosa* etc (Bhatnagar and Kim, 2011; Saadoun *et al.*, 1999; Selvameenal *et al.*, 2009 and Servin *et al.*, 2008).

Actinomycetes also synthesize and excrete dark pigments, melanin or malanoid (Zenova 1965; Arai and Mikami 1972; Amal *et al.*, 2011). These melanin compounds are irregular, dark brown polymers having radio protective and antioxidant properties that can effectively protect the living organisms from ultraviolet radiation (Romero-Martinez *et al.*, 2012). Melanins are frequently used in medicine, pharmacology and cosmetics preparation (Quadri and Asgar, 2012).

Rhizosphere soil of these plants may be an attractive actinomycetes source, able of producing novel secondary metabolites. Catalase, Chitinase and Urease enzymes also produce from actinomycetes (Wutthithamavet, 1997).

Materials and Methods

The present work has been undertaken to study the “Screening and Isolation of Pigment Producers and Non-pigment Producers Actinomycetes from Rhizospheric Soil Sample”. The experiments were carried out adopting the following Materials and Methods.

Collection of soil sample

Rhizospheric soil sample were collected from various medicinal plant. Total 8 soil samples were screened for isolation of Actinomycetes.

These sample were mainly collected from various region of Karanja (Lad) Dist. Washim. The soil samples were collected from a depth of 10 inches from surface area in commercially available sterile polythene bags.

Pre-treatment of the soil sample

The soil samples were pretreated in order to reduce the proportion of other microorganisms than Actinomycetes. The soil samples were dried for about 50 – 60°C for 5 – 10 minutes (Selvamohan *et al.*, 2016).

Isolation of actinomycetes

Each soil sample (1g) was suspended in 100 ml sterile distilled water, then homogenized by vortex mixing, mixtures were allowed to settle and serial ten folds dilutions up to 10^{-4} were prepared by using sterile distilled water. Isolation was carried out on Actinomycetes Isolation agar plates by spreading. The plates were incubated at 37°C for 24 hrs. After attaining a powdery growth, the colonies were observed, based on the colour and Morphological differences, colonies were selected and restreaked on Actinomycetes Isolation Agar medium. Further the culture is preserved for antagonistic studies (T. Selvamohan *et al.*, 2016).

Characterization of actinomycetes

The potent Actinomycetes isolates selected from primary screening were characterized by Morphological, biochemical and physiological method. The morphological method consists of macroscopic and microscopic characterization. Macroscopically the Actinomycetes isolates were differentiated by their colony characters, e.g. size, shape, colour, consistency etc. For the microscopy, the isolates were grown by cover slip culture method (Kawato and Sinobu, 1979).

In vitro screening of Isolates for antagonism

Actinomycetes isolates were tested for their antibacterial activity against bacterial pathogens namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus*. The antibacterial activity was carried out by Agar Well diffusion method. The Actinomycetes isolates isolated from the rhizosphere soil of medicinal plants were spotted on the pre-poured nutrient agar plates already having a lawn of indicator test bacteria on it. Plates were incubated at 37°C for 24 hrs and observed for clear zone formation around the well. Antibacterial activity was expressed in terms of diameter (mm) of clear zone produced around the well at 37°C for 24 hrs. (Mohinder Kaur *et al.*, 2015).

Antibiotic susceptibility test

The antibiotic sensitivity of test strains was determined by the standard Disc diffusion method against a number of antibiotics. All antibiotic discs were purchased from the Himedia Pvt, Ltd. (Bombay, India). The antibacterial activity was performed according to CLST, USA guidelines on Mueller Hinton Agar Well Medium using diffusion method (Anitha *et al.*, 2009; EI-Mehalawy *et al.*, 2005).

Partial purification of the pigments from actinomycetes

The natural pigment producing Actinomycetes colonies obtained in the present study were inoculated in 100 ml of yeast extract broth and incubate at 37°C for 24 hrs. After incubation the pigmented broth was centrifuged at 2000 rpm for 15 min and the supernatant was recovered. This step was repeated twice (Velmurugan *et al.*, 2009). Supernatant taken out and measure the optimum density at various wavelength range.

Results and Discussion

In the present study 8 actinomycetes strains were isolated from Rhizospheric soil sample of different medicinal plants from various region of Karanja (Iad) Dist. Washim. It was based on the colony Morphology and Pigmentation.

Isolation of Actinomycetes has always been faced with difficulties in comparison to their competitors like other bacteria (William and Corss, 1971).

All the obtained isolates were found to be Gram negative organism. However, results reported by Taradevi Gurung *et al.*, (2009) showed that both Gram negative and Gram positive bacteria were obtained during their study. This might be due to the morphological difference between those two types of microorganisms (Nakaido and Vaara, 1985).

Out of 8 isolates two were found to be pigment producers Actinomycetes i.e. isolate A2 and H. Isolate A2 shows pink colour whereas isolate H shown bluish green colour on Actinomycetes Isolation Agar. According to Teli *et al.*, (2000) reported that in their study the pigments such as pink, yellow, deep pink, greenish brown and brown diffused in the Kuster's broth were partially purified.

The cultural characters of the Actinomycetes strain T1, T2, A1, A2, N, H, Tu, and Ni on Actinomycetes isolation agar medium were studied. It was noticed that Actinomycetes Isolation Agar medium favoured the rapid growth of powdery colonies within incubation period at 37°C for 24 hrs. Similarly Nakaido and Vaara (1985) showed that the screening revealed that more isolates were active against gram positive bacteria than Gram negative bacteria. The biochemical study indicates that Actinomycetes, isolates showed fermentation of Lactose and Maltose with acid and gas production where as in case

Glucose and mannitol rest of other isolates shows only acid production but no gas production. During the biochemical studies it was noticed that Methyl Red Test was positive for all the isolates except for isolate N whereas all isolates utilizes citrate showing positive test. Beside this only two isolates i.e. A1 and A2 shows VP test positive and indole test was found to be negative for all isolates.

The enzyme production ability of isolates was studied. The results revealed that actinomycetes shows positive catalase and oxidase activity whereas only two N and H isolates shows gelatin liquification, According to Martin *et al.*, (2008) the biochemical studies indicate that all Actinomycetes, isolates can be used a potent source for the enzymes like amylase, lipase, caseinase and gelatinase. The enzymes are very much useful for their saprophytic mode of life.

Many actinomycetes have been isolated from various natural sources, as well as in plant tissues and rhizospheric soil Biological function of Actinomycetes mainly depend on sources from which the bacteria are isolated (Sharmin, 2005).

Totally 8 Actinomycetes isolates were tested against 5 different antibiotics *i.e.* Amoxicillin (10 mg), Erythromycin (15 mg), Tetracycline (30 mg), Ciprofloxacin (10 mg), Amoxyclave (30 mg) and the results were recorded in terms of zone of inhibition in mm. The most surprising result was obtained in case of Ciprofloxacin. Ciprofloxacin found to be sensitive against all isolates and shows very excellent zone of inhibition ranging from 18 – 27 mm.

However, Amoxicillin is sensitive only against A1 isolates and for rest of isolates it was resistant. Similar pattern of result was observed in case of Erythromycin which was found to be sensitive only against isolates A1 and Ni and shows resistant towards other isolates.

Amoxyclave shows intermediate type of activity but active against isolate A1. Hence overall results revealed that all the antibiotics were sensitive against isolate A1 obtained from rhizospheric soil of Alovera.

Antibiotics susceptibility pattern were tabulated (Table 1, Graph 1). From the above results Ciprofloxacin antibiotic showed excellent activity. Erythromycin and Tetracycline showed good to moderate activity whereas Amoxycillin and Amoxyclave showed no activity. According to Sandepta *et al.*, (2015) that the Bacillus strain were observed to inhibit growth of Gram positive and Gram negative test organisms such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp.*, and *Pseudomonas spp.* indicating that antibiotic produced by strains is a broad spectrum antibiotic. Interestingly, the isolated strain showed resistance to tetracycline.

Antibacterial activities were performed by disc-diffusion assay and effectiveness was measured by zone of inhibition on bacterial culture plates.

The isolates Actinomycetes spp. against test bacteria was tabulated in Table No. 2, from the above result *S. aureus* showed activity for T2 isolate showing zone of 30 mm and very less activity (6 mm) for Ni isolate was observed. *E. coli* showed moderate type of activity for all the isolates. In case of *P. aeruginosa* best activity was shown by the N and H isolate *i.e.* 25 mm and 28 mm respectively and for rest of other isolates moderate activity was shown by the same organism. It was noticed that isolate N and H again shows excellent activity towards *P. vulgaris*.

From the complete observation it was noticed that Isolate N obtained from Neem soil and H from Hibiscus soil is having excellent

antagonistic activity in controlling all the test pathogens. Arifuzman *et al.*, (2010) studied soil samples of Karanjal region of Sundarbans of Bangladesh and 55 actinomycetes were isolated and screened for antibacterial

activity. Out of 55 isolates 20 isolates (36.36%) were active against the test organisms. Dehnad *et al.*, 2010 studied the antibacterial activity of *Streptomyces* isolates from soil samples of west of Iran.

Table.1 Antibiotic susceptibility pattern of obtained isolates

Isolates	Zone of inhibition (diameter in mm)									
	Amoxycillin		Erythromycin		Tetracycline		Ciprofloxacin		Amoxyclave	
T ₁	12 mm	R	13 mm	R	15 mm	I	20 mm	S	16 mm	I
T ₂	--	-	10 mm	R	10 mm	R	26 mm	S	--	-
A ₁	26 mm	S	26 mm	S	23 mm	S	26 mm	S	22 mm	S
A ₂	--	-	12 mm	R	10 mm	R	24 mm	S	--	-
N	12 mm	R	14 mm	R	15 mm	I	25 mm	S	16 mm	I
H	--	-	10 mm	R	--	-	27 mm	S	--	-
Tu	11 mm	R	12 mm	R	16 mm	I	18 mm	S	10 mm	R
Ni	--	-	23 mm	S	16 mm	I	21 mm	S	--	-

Where, -- = No zone of inhibition, R = Resistance, S = Sensitive, I = Intermediate

Table.2 Isolates strains of actinomycetes activities against pathogenic bacteria *i.e.* *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris*

Isolates	Zone of inhibition (diameter in mm)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>P. vulgaris</i>
T ₁	15 mm	10 mm	15 mm	10 mm
T ₂	10 mm	30 mm	10 mm	8 mm
A ₁	16 mm	5 mm	1 mm	1 mm
A ₂	1 mm	11 mm	10 mm	10 mm
N	15 mm	15 mm	25 mm	20 mm
H	21 mm	6 mm	28 mm	22 mm
Tu	5 mm	15 mm	15 mm	20 mm
Ni	3 mm	6 mm	24 mm	4 mm

Table.3 Optical density of two pigment producer Actinomycetes at different ranges

Isolate	Wave length (nm)					
	420	440	520	560	620	660
A2	0.762	0.689	0.476	0.342	0.342	0.332
H	1.184	1.032	0.648	0.570	0.492	0.490

Fig.1 Antibiotic susceptibility pattern of obtained isolates

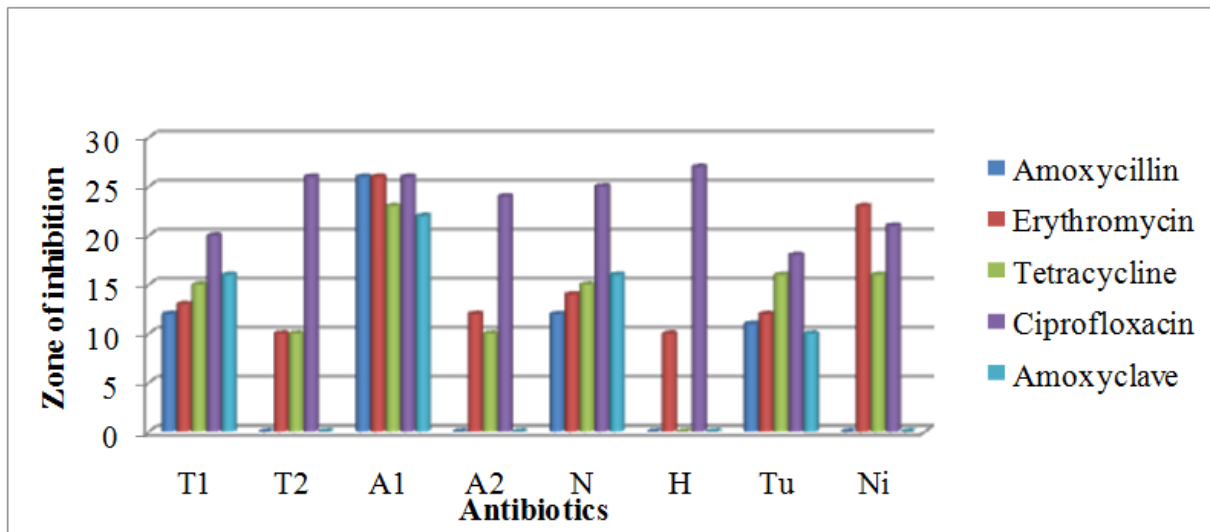


Fig.2 Activities of isolated actinomycetes strains against test bacteria

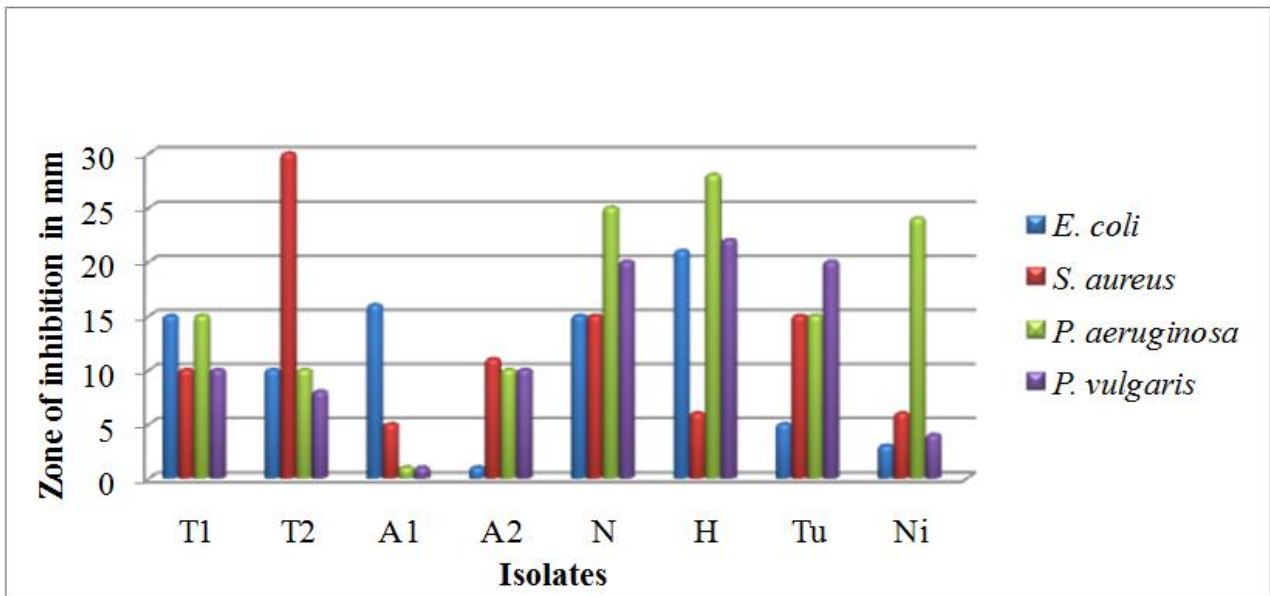
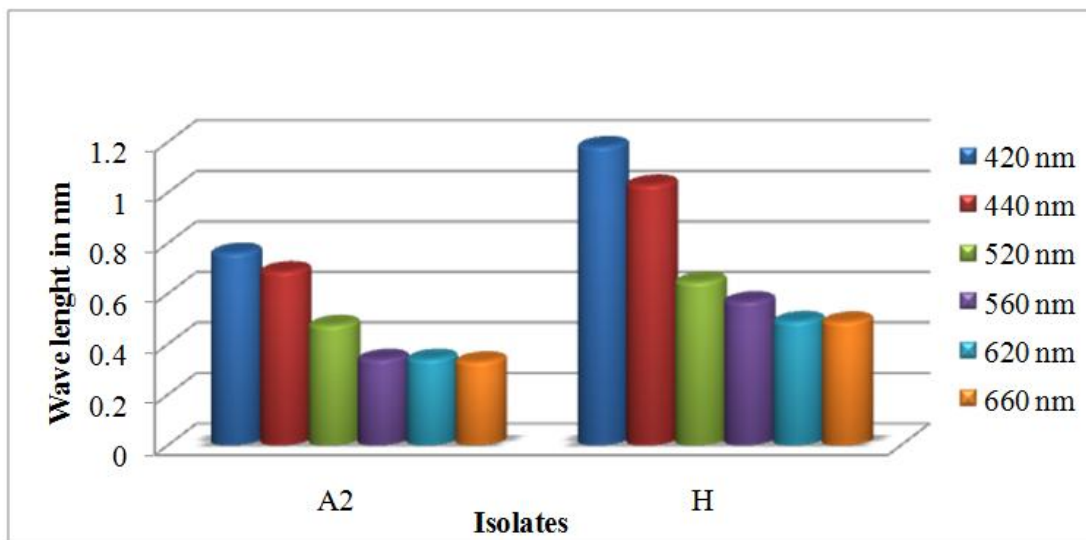


Fig.3 Optical density of two pigment producer Actinomycetes at different ranges



Zakir *et al.*, (2002) showed that the antibacterial activities were studied by well plate method. All the Actinomycetes isolates showed antibacterial activity against five pathogenic organisms.

Similarly in previous study Jarallah (2014) isolated *Streptomyces* KLG 13 from agricultural soil in Hillah city that possess high antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *Marganella spp.*

The Actinomycetes isolates showed pigmentation of optimum density measure at all wave length. Table No. 3 leads to Optimum density results (Fig. 3). It was observed that for Isolate H higher activity was observed at all wave length. The highest activity was measured at 420 nm followed by 440 nm for isolate H. Other isolate showed moderate activity. Very less activity was shown by A2 isolate for 560, 620 and 660 nm

Teli *et al.*, (2000) stated that the production and evaluation of microbial pigments as textile colourants is currently being investigated. Similarly Ramasamy Amsaveni *et al.*, (2015) reported that natural colours or

biocolours have numerous applications in textile as well as in food industries as colouring agents.

Further characterization of Actinomycetes and their products for utilization in Plant Biotechnology, Urban Waste Management, Environmental Biotechnology and some other applications yet to be done, the potential number of metabolites from Actinomycetes may be discover in future.

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