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Isolation and screening of antibiotic producing actinomycetes from soilsin Manong, Perak, Malaysia

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ABSTRACT

The present study focuses on isolation of actinomycetes from the soil in Ulu Kenas forests of Manong, located in the northern part of Perak state in Malaysia and screening for antibacterial activity. All the isolates were screened for their antibacterial activity using Kirby-Bauer (KB) method against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pnemoniae and Staphylococcus aureus. Out of the ten isolated actinomycetes, seven, namely B, D, E, F, H, I and J showed antimicrobial activity against selected bacteria. However, isolates E showed maximum inhibition against P. aeruginosa and the isolates I and J against K. pneumonia with highest scores. The findings of the study may be helpful to the future investigators in identifying alternative and new bioactive secondary metabolites like antibiotics to treat resistant human pathogens.

Keywords: Soil *actinomycetes*, Antibacterial activity, Tryptic Soy Broth, Bioactive compounds.

INTRODUCTION

Bacterial resistance to antibiotics has emerged as a serious concern in recent years which has attracted the researchers to search for newer antibiotic substances that would help in combating bacterial diseases [1]. *Actinomycetes* are believed to be an excellent source of producing antibacterial substances with high commercial value. They are prokaryotes of Gram-positive bacteria with a distinguished morphological feature from other bacteria and tend to grow slowly as branching filaments, found in nature in soils, ponds, plant residues and food products [2]. More than 70% of naturally occurring antibiotics are believed to be isolated from different genus of *actinomycetes* [3]. Extensive literature survey revealed that there are no reports on antibiotic producing *actinomycetes* from the soil samples collected in Ulu Kenas forests of Perak state, Malaysia. Thus, the objective of the present study was to isolate antibiotic producing *actinomycetes* from the soil samples in Ulu Kenas forests.

MATERIALS AND METHODS

Sampling

The study area was located in Ulu Kenas forests of Manong located in the northern part of Perak state. Prior to beginning of our research, appropriate permission was obtained from Perak Forestry Department and the soil samples were collected from randomly selected 10 different sites. The collected soils samples were preserved in separate plastic bags and labelled from A to J. Information on the sampling such as the location, type and condition of the soil, colour and pH of the soil was recorded for every soil sample taken. The collected samples were preserved in refrigerator at 2°C until further use.

Isolation of *actinomycetes*

The collected soil samples were passed through 250 µm pore size mesh to remove large debris and dried at 45°C for 12 h. Following drying, 1 g of each soil sample was separately mixed with 100 mg calcium carbonate and incubated at 37˚C for 7 days in an incubator. The soil samples were added in different test tubes containing 10 ml of 0.85%

physiological saline solution and homogenized followed by centrifugation at 500 rpm for 15 min. The test tubes were marked as stock cultures (10^{-1} concentration) for different soil samples. A volume of 1 ml was transferred separately and aseptically from the stock cultures, to other test tubes containing 9 ml of sterile 0.85% physiological saline solution. The mixture was further diluted to another test tube by taking 1 ml of aliquot of the mixture and mixed with another 9 ml of sterile 0.85% physiological saline solution. This mixing resulted in to 10^{-2} dilution factor. The process of serial dilutions were continued to get dilutions up to 10^{-6} for all soil samples. All the samples were incubated for overnight. After incubation, 100 µl of the suspension from each culture tube were spread evenly with a sterile spreader over the surface of sterile Tryptic Soy Agar (TSA) media aseptically using spread plating technique. Amoxicillin(20 µg/ml) and fluconazole (25 µg/ml) were added in to the media to prevent bacterial and fungal growth respectively. The plates were then were incubated aerobically at 37˚C for 7 days. All the samples then were observed intermittently during the incubation period [4, 5].

During the incubation, observation was made to identify actinomycetes on the plates depending on their color and nature of the colony on the media. The pure colonies were isolated and further identified by the colour of hyphae, smell, morphology and the presence or absence of aerial and substrate mycelium. The identified colonies were then transferred to other plates containing sterile TSA medium and incubated at 37 °C for 7 days. After incubation, they were subcultured and purified by streaking to the nutrient agar (NA). After purification, the isolates were preserved at 4°C for further studies [6].

Preliminary screening of crude antibiotic

Agar streak method

The isolated actinomycetes were screened for their antibacterial activity using Kirby-Bauer (KB) method against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC10145, *Klebsiellapnemoniae* ATCC 13883and *Staphylococcus aureus* ATCC 9144.In this test, discs containing different isolates were placed on an agar plate where the selected bacteria were grown. Sterile culture solutions prepared in normal saline were spread on sterile nutrient agar plates using spread plate technique. A500 µl of the broth of each isolate was centrifuged at 9000 rpm for 20 min. The supernatant (15 µl) was pipetted on the sterile blank disc and placed over the selected bacterial strain. A sterile blank disc containing 15 µl of Tryptic Soy Broth (TSB) served as negative control and ciprofloxacin(20 µg/ml) as positive control. The plates were then incubated for 24 h at 37˚C. After incubation, the zone of inhibition was recorded. Based on their antimicrobial properties, isolates were chosen for the further biochemical characterization [7].

Morphological characterization

Morphological characters of the actinomycetes strains such as type of areal hyphae, growth of vegetative hyphae, colony characteristics, fragmentation pattern and spore formation were studied by inoculating the selected strain into sterile TSA media by streak plate method [7].

Microscopic characterization

Microscopic characterization of the actinomycetes was performed using Gram staining method [8].

RESULTS

Isolation of actinomycetes

A total of 10actinomycetes were isolated from the soil samples. The isolated strains were characterized with pinpoint colonies. The details of the soil samples and the number of actinomycetes on isolation media are presented in Table 1.

Preliminary screening of crude antibiotic

Out of the ten isolated actinomycetes screened for antimicrobial activity, seven, namely B, D, E, F, H, I and J showed antimicrobial activity against selected bacteria (Table 2). However, isolates E showed maximum inhibition against *P. aeruginosa* and the isolates I and J against *K. pneumonia* with highest scores.

Morphological characterization

Morphologically, the colonies are mostly cream coloured and moist. The microscopic characterization after Gram staining revealed that the isolate E, I and J are Gram-positive and rod-shaped microorganisms. All the strain belong to the genus Actinomycetes.

Table 1: Characteristics of soil samples and number of actinomycetes isolated

Sample	Dilution of Sample	No of Actinomycetes on isolation media	Nature of soil sample	Soil pH
A	10^{-4}	>100	Near river, under tree	5
	10^{-5}	50		
	10^{-6}	5		
B	10^{-4}	35	Under tree, road side	5.5
	10^{-5}	11		
	10^{-6}	4		
C	10^{-1}	Nil	Uphill, under tree	5
D	10^{-4}	>100	Uphill, under tree	
	10^{-5}	>100		
E	10^{-4}	54	Higher up hill, under trees	5.5
	10^{-5}	16		
	10^{-6}	3		
F	10^{-4}	>100	River side	5.7
	10^{-5}	62		
	10^{-6}	5		
G	10^{-4}	>100	River side	5
	10^{-5}	16		
	10^{-6}	7		
H	10^{-4}	19	River side	5.5
	10^{-5}	14		
	10^{-6}	3		
I	10^{-4}	50	Near river, besides anthill	5.7
	10^{-5}	6		
	10^{-6}	3		
J	10^{-4}	12	Near river	5.5

Table 2: Sensitivity of various microorganisms to the soil isolates

Sample	*S. aureus*	*E.coli*	*P. aeruginosa*	*K. pneumoniae*
B	-	-	-	+
D	-	-	-	+
E	-	-	+++	+
F	++	+	-	+
H	+	++	-	+
I	+	+	-	+++
J	-	-	-	+++

+++ = Very good inhibition, ++ = Good inhibition, + = Moderate inhibition, - = No inhibition

DISCUSSION

Actinomycetes are anaerobic, Gram positive bacteria that tend to grow slowly as branching filaments. There are several species of *Actinomycetes* occur in soil and their bioproducts have been a phenomenal success [9]. Bioprospecting for new leads are often compounded by the recurrence of known antibiotics in newer microbial isolates [10]. The research that had been done towards the discovery of some antibiotics from the soil across the globe led us to come out with the research on antimicrobial agents in soil in Malaysia.

In the present study, ten actinomycetes were isolated from soil samples in Ulu Kenas forests of Manong, Perak, Malaysia. Calcium carbonate was used in the treatment of the soil to stabilize the pH of the soil which was then ready for the isolation. El-Nakeeb and Lechevalier [11] reported that treatment of soil samples with calcium carbonate offers higher total and relative plate counts of actinomycetes[11].

CONCLUSION

The findings of the study may be helpful to the future investigators in identifying alternative and new bioactive secondary metabolites like antibiotics to treat resistant human pathogens.

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REFERENCES

[1] Antimicrobial resistance: Global Report on Surveillance. (**2014**). World Health Organization, Geneva (Available online http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf)

[2] C.Subathra Devi, A. Kumari, N. Jain, S.JemimahNaine, V.Mohanasrinivasan, *Int. J. Pharm. Pharm. Sci.*, **2013**,5(4), 483-489.
[3] A.Bizuye, F.Moges, B.Andualem,*Asian Pac. J. Trop. Dis.*,**2013,** 3(5), 375-381.
[4] K. Narendra, K. S. Ravi, S. K.Mishra, A. K.Singh, U. C. Pachouri, *Int. J.Microbiol. Res.*,**2010**,2(2), 12-16.
[5] A. L. T.Kalyani, K. M.RamyaSravani, J. Annapurna, *Int. J. Curr. Pharm. Res.*,**2012**, 4(2), 109-112.
[6] M. Kashif, V. Kumar, V. N.Kalpana, V. D.Rajeswari,*Der Pharmacia Lettre*, **2016**, 8(1), 16-24.
[7] B. K.Nanjwade, S. Chandrashekhara, M. Ali, P. S.Goudanavar, F. V. Manvi, *Trop. J. Pharm. Res.*, **2010,** 9(3), 231-236.
[8] H. S.Chaudhary, J. Yadav, A. R. Shrivastava, S. Singh, A. K. Singh, G.Natrajan, *J. Adv. Pharm. Tech. Res.*, **2013**, 4(2), 118–123.
[9] B. Kumari, R.Vijayabharathi, V. Srinivas, S.Gopalakrishnan, *African J. Biotech*., **2014,**13(26), 2582-2592.
[10] G. B. Mahajan, L. Balachandran, *Front. Biosci., (Elite edition)*,**2012**, 4, 240-253.
[11] M. A. El-Nakeeb, H. A. Lechevalier, *Applied Microbiol*.,**1963**, 11, 75-77.