Environment & Ecology 29 (1A) : 473—476, 2011
 ISSN 0970-0420

Production of Potent Antibiotic from Actinomycete Isolated from Soil

PUNYA, JAYASHRI GHOSH, DINKAR, JYOTSANA K. PETER AND ASHUTOSH SHUKLA

Microbiology Laboratory College of Biotechnology & Allied Sciences AAI (DU), Allahabad, India
E-mail : punyamsingh@gmail.com

Abstract

Micro-organisms were isolated from soil samples from different localities of Allahabad, UP and screened for production of antibiotics. Six strains were selected to establish their antimicrobial / antibacterial activity against test organisms. These isolates showed various degrees of antimicrobial activity. Finally one potent isolate A_1 was selected which was isolated from rhizosphere of jatropha plant. It showed the maximum zone of inhibition and thus the maximum antibiotic production. Different pathogens viz. *Salmonella typhi, Bacillus subtilis, Shigella dysentriae, Micrococcus, Staphylococcus aureaus* and *Escherichia coli,* were used for screening to observe the antibacterial activity of strain A_1. The novel antibiotic produced from strain A_1, was most effective against *S. typhi* on other hand *Bacillus subtilis* was least inhibited.

Key words : Actinomycete, Antimicrobial activity, Test microorganisms, Inhibition zone.

The discovery and development of antibiotics has been hailed as one of the greatest contribution to science and technology. Antibiotics are indispensable weapon in the medical armoury. From practical point of view many of the most dreadful diseases with high mortalities have now been brought under control by them. The potentialities of antibiotics have not yet been fully realized and thousands of scientists and workers are busy all over the world examining microorganisms as potential source of new antibiotics. Antibiotics are produced by certain fungi, bacteria, actinomycetes, algae and lichens. About 800 antibiotics from fungi, about 400 from bacteria and over 2000 from actinomycetes, have been discovered so far (1—8). Among them most important and fertile source of commercial important antibiotics is the genus *Streptomyces*. Antibiotic resistance has become a major problem for doctors and researchers and the people that may one day need to take an antibiotic. The best source for obtaining new antibiotics is from soil inhabiting microorganisms. The emergence of clinical bacterial strains exhibiting resistance against conventional antibiotics has urged the search for novel antibiotic agents. Even though there are number of antibiotics produced and marketed globally the thirst for identification and screening of new antibiotics become unquestionable. Therefore the aim of work was to investigate antibiotic producing microorganisms from soil samples of Allahabad city of UP, evaluation of their antimicrobial activity and identification of promising isolates.

Methods

Soil samples were obtained from various

Table 1. Enumeration of plate count and antibiotic producing colonies from different soil samples.

Sample no.	Sources	Plate count (cfu/ml)	No. of colonies producing antibioticts	Strain designation
1	Neem plant	4.93×10^7	Nil	
2	Railway track	2.58×10^7	Nil	
3	Jatropha plant	8.63×10^7	3	A_1, A_2, A_3
4	Moist soil	1.63×10^7	Nil	
5	Arhar field	2.52×10^7	1	A_4
6	Pea field	1.30×10^7	2	A_5, A_6

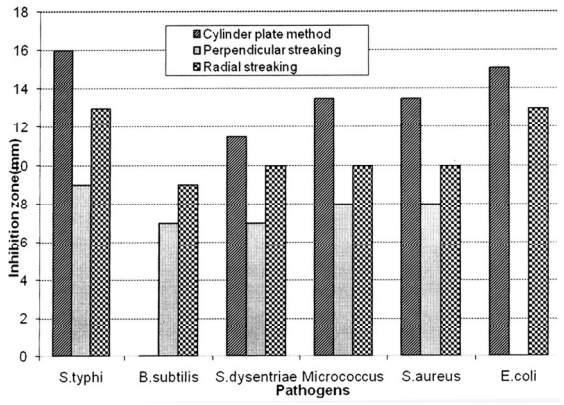

Figure 1. Inhibition zone obtained by different screening method for antibacterial activity of antibiotics produced by strain A_1.

sources viz. neem plant, jatropha plant, railway track, arhar field, pea field and moist soil of different location of Allahabad. The colonies obtained from these soil samples were screened for antimicrobial / antibacterial activity. The colonies were isolated from soil by serial dilution method on nutrient agar plate.

Preliminary Assay

The colonies obtained on nutrient agar plate were covered with sterilized filter paper. The nutrient broth containing unknown bacterial culture mixed with molten nutrient agar and spread over the filter paper. The plates were incubated aerobically at 30 C for 48 hours. Colonies producing inhibition zones were isolated.

Table 2. Morphological characteristics of colonies of antibiotic producing strains on nutrient agar.

Strain designation	Colony characteristics
A_1	White colored, round, entire margin, elevated opaque, dry
A_2	Pale yellow, lobed margin, translucent, round flat
A_3	Pale yellow, irregular margin, light colored center, flat, discrete colony
A_4	White, gummy, irregular margin, flat
A_5	Very small gummy colony, round with wavy margin
A_6	Pale yellow color, round, mucoid, opaque

Table 3. Confirmatory filter paper method for final selection of colony.

Strain designation	Inhibition zone (mm)
A_1	17
A_2	7
A_3	5.5
A_4	6
A_5	6.2
A_6	9

Confirmatory Assay

The point inoculation of isolated colonies was done on nutrient agar plates. Each colony was covered with sterilized filter paper and molten nutrient agar was poured over the filter paper. The culture of pathogenic bacteria was swabbed on each solidified nutrient agar which was solidified on filter paper. The plates were incubated aerobically at 37 C for 48 hours. Colonies producing inhibition zones were isolated.

Screening for Antimicrobial Activity

A number of screening methods viz. cylinder plate method, perpendicular streaking and radial streaking were employed to observe the antibacterial activity of the strain. Different pathogens viz. *Salmonella typhi, Bacillus subtilis, shigella dysentriae, Micrococcus, Staphylococcus aureaus and Escherichia coli* were used for screening.

Perpendicular Streaking

The pure culture of the isolate was streaked across one side of nutrient agar plate and incubated at 37 C temperature. Culture of six test organism were the streaked at right angles to the isolates, starting the inoculation at the edge of isolate and working away from it.

Table 4. Antibiotic production of strain at different time duration assayed by the zone of inhibition.

Strain designation	Time (h)	Inhibition zone (mm)
A_1	24	5
	48	Poor inhibition
	72	No inhibition

Radial Streaking

Point inoculation of the isolate at the center of nutrient agar plate was done and incubated at 37 C. The culture of six test organisms were streaked from the edge of colony to the edge of plate like spoke of a wheel. The plate was incubated at optimum temperature of the test organisms.

Cylinder Plate Method

The isolate was inoculated at the center of potato dextrose agar plates and incubated at 37 C. Six plates of nutrient agar were swabbed with six pathogenic cultures. In every nutrient agar plates wells (5 mm) were cut with the help of cork borer. Six plugs were cut from PDA plate containing colony of isolate and placed into the wells of nutrient agar and incubated at 37 C for 24 to 48 hours and the zone of inhibition was measured.

Cup Plate Method

Pure colonies were inoculated in MGY media (g/liter in distilled water ; glucose (1.0) ; yeast extract (0.1) ; $(NH_4)_2$ SO_4 (2.0) ; K_2HPO_4(0.25) ; $MgSO_4$ (0.25) ; KCl (1.0) ; Agar (17.0) ; pH (3.5–4) and incubated for interval of 24 hours. The nutrient agar plates were swabbed with known pathogenic culture and then cups were cut with the help of cork borer. The antibiotic producing organism was put into cups. The plate was incubated the higher concentration of antibiotic producing organism the greater the zone of inhibition.

The isolate being an actinomycetes was determined by micro slide culture technique and growing them on actinomycetes medium. With the help of sterilized flamed inoculation scalpel, area of 1 square centimeter of the nutrient agar medium was cut and transferred to slide and located centrally in another sterilized petri plate and then blocks were covered with coverslips. The isolates were inoculated at four corners of agar medium. The plates were incubated at 37 C for 24—48 hours. The point inoculation of the sample isolate was done on actinomycetes medium (g / liter in distilled water ; glucose (4.0) ; yeast extract (4.0) ; malt extact (10.0) ; $CaCl_2$ (2.0) ; agar (17.0) ; pH (7.2) and incubated at 37 C for 24—48 hours.

Results and Discussion

The six isolates showed considerable antimicrobial activity in preliminary assay of filter paper method (Table 1). The morphological properties of the selected isolates were studied (Table 2). To investigate the best antibiotic producing micro-organism confirmatory assay was done. It was observed that out of six strains from three soil samples only one strain (A_1) which was isolated from jatropha plant showed maximum zone of inhibition thus maximum antibiotic producing organism (Table 3). By using cup plate method it was observed that on increasing the time of incubation i.e., from 24 to 72 hours, the effect of antibiotics was found to be reduced (Table 4). Microslide culture technique gave the indication of the strain being actinomycete. The growth obtained on Actinomycetes media confirmed that the isolate which show the maximum antimicrobial activity is an actinomycete. Rangaswami and Bagyaraj (1) have also reported novel antibacterial antibiotics from soil.

It was observed that our novel antibiotic was most effective against *S. typhi*, on the other hand *Bacillus subtilis* was least inhibited by the antibiotic (Fig. 1).

Soil was chosen as the source of antibiotic producing microorganisms as most of the microorganisms, which produce antibiotics, live in soil. This is due to the phenomenon of antibiosis, their growth, nutrition and survival value are enhanced in competitive world of microflora of the soil.

References

1. Rangaswami G. and D. J. Bagyaraj. 1998. Agricultural microbiology. 2nd edition. Prentice Hall of India Pvt. Ltd., New Delhi, India 165—395 pp.
2. Boman H. G. 1995. Peptide antibiotics and their role in innate immunity *Ann. Rev. Immun.* 13 : 61—92.
3. Bruggink A. and P. D. Roy. 2001. Industrial synthesis of semisynthetic antibiotics. Pb. 12—54. *In* A. Bruggink (ed). *Synthesis of B-lactem antibiotic*s. Dordrecht : Klawar.
4. Shete K. and V. C. Vora. 1956. Screening of India soil for antibiotics and vitamin B_{12} producing microorganisms. *Symp.* Hindustan Antibiotic (Pri-

vate) Ltd. Pimpri. 5—9 pp.
5. Sadasivan R. and J. N. Tayal. 1956. Biolological assay of antibiotics. Sympos. Hindustan Antibiotic (Private) Ltd., Pimpri. 120—127 pp.
6. Prescott L. M., J. P. Harley and D. A. Klein. 2005. Antimicrobial chemotherapy. Pp. 79—98. *In Microbiology.* 6th edition Mc Graw hill, London, UK.
7. Jackson G. G. and H. F. Dowling. 1953. Adverse effect of antibiotic treatment. *Gen. Pract.* 8 : 34—40.
8. Humphrey J. H. and J. W. Lightbown. 1955. A general theory for plate assay of antibiotics with some practical applications. *J. Gen., Microbiol.* 7 : 120—143.