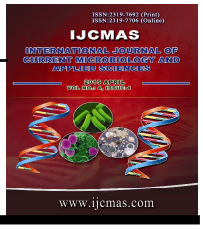
Int.J.Curr.Microbiol.App.Sci (2015) 4(4): 123-126

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 4 (2015) pp. 123-126**
http://www.ijcmas.com



Original Research Article

Studies on Isolation of Antimicrobial Actinomycetes from Osmanabad Soil

S.G.Pujari, N.R.Kadam, S.G.Chaudhari*, P.P.Dixit and A.M.Deshmukh

Department of Microbiology, Dr. Babasaheb Ambedkar Marathwada University,
Sub campus, Osmanabad-413501 (M.S.) India
**Corresponding author*

K e y w o r d s

Actinomycetes,
Giant streak,
Well diffusion

A B S T R A C T

Thirteen isolates of actinomycetes (designated A to M alphabetically) were obtained from soil samples collected from Osmanabad (M. S.). All the isolates were screened primarily for antimicrobial potential by giant streak method on glycerol asparagine agar. The antimicrobial activities were tested against 13 human pathogens. All strains of actinomycetes showed inhibitory activity against *S. auerus, Proteus* and *S. fecalis.* Actinomycetes strain D and E were showing highest antimicrobial spectrum thus can carry further for fermentation using 100 ml glycerol asparagine broth on rotary shaker in 150 ml conical flask at 37^0C for 5 to 6 days. During secondary screening the fermentation broth centrifuged at 4000 rpm for 20 minute and antimicrobial activity were assayed by using well diffusion method.

Introduction

As per the rule of adaptation, currently existing pathogens showing increased resistance against antimicrobial compound available today (Amit Pandey *et al.*, 2011). To face this problem it is essential to search out the antimicrobial potentials of the existing strains that is why the current work deals with the study of antimicrobial potential of Actinomycetes. The antimicrobials are the substances secreted by certain microorganism as a secondary metabolite which show inhibitory effect against certain other strain of microorganism (Gunasekaran Mohanraj and Thangavel Sekar, 2013). Antibiotics are potential antimicrobials and actinomycetes are the predominant species producing antibiotics (Gunasekaran Mohanraj and Thangavel Sekar, 2013). The actinomycetes are Gram positive bacteria with high G+C content. Major actinomycetes are aerobic spore forming filamentous organism dominantly present in soil. In present work the Actinomycetes strain were isolated from Osmanabad and nearby area soil and screened for their antimicrobial activity (Abebe Bizuye *et al.*, 2013; El-Khawaga and Megahed, 2012; Bhagabati Pandey *et al.*, 2005).

Material and Methods

Sample collection

The soil samples were collected in a polythene bag and carried up to the lab from

the Ayurvedic college garden Osmanabad, Tuljapur farm district Osmanabad, and Dr. Babasaheb Ambedkar Marathawada University sub campus, Osmanabad (Abebe Bizuye *et al.,* 2013; El-Khawaga and Megahed, 2012; Bhagabati Pandey *et al.,* 2005) and kept in the refrigerator.

Isolation

1 gm soil sample from each sample were mixed in 25ml distilled water which were taken in the 15ml conical flask and kept on rotary shaker for 30 minutes after that these flask were taken out from rotary shaker and were prepared 10 fold dilution of each flask. 0.1ml sample from 10^{-2} were spread upon nutrient agar plates and plates were incubated at 37^0C for 5–6 days.

Primary screening

Antimicrobial activity of selected strain carried out by perpendicular streak plate method or also called gaint streak method on glycerol asparagines agar.

The plates with ribbon like full growth of selected strains after 5 to 6 days of incubation at 37^0C were utilized to screen out the inhibition spectra of it against test organism. The test organisms are *E. coli 142, E. coli 121, Klebsiella (MDR), Granulicatella, Providencia stuartii, S. fecalis, Candida albicans* these are collected from Shri Shivaji Mahavidhyalaya, Barshi. Then *E. coli, Klebsiella, Proteus, Pseudomonas* and *S. auereus* collected from SBZ College, Barshi (Amit Pandey *et al.*, 2011)

Culture maintenance

All the isolates were slanted on to sterile glycerol asparagine agar medium for pure culture maintenance and abbreviated as A,B.C,D,E,F,G,H,I,J,K,L,M.

Secondary screening

Two strains D and E showing highest spectra were cultivated in fermentation media on shaker for the experiment extracted using ethyl acetate with shaking it for 1 hr. and concentrated in methanol, and then this was used for well diffusion method that is secondary screening

Inoculum preparation

Glycerol asparagine broth was used as the inoculation media, and selected strain inoculated in to the sterile glycerol asparagine broth incubated at 37^0C on rotary shaker in 150 ml flask for 48h

Fermentation media

Glycerol asparagine broth was used as the fermentation media. 10% of 48h old inocula were transferred aseptically into 100 ml fermentation media and is incubated at 37^0C for 5 to 6 days on rotary shaker in 150 ml conical flask. Then the culture broth was centrifuged at 4000 rpm for 20 min and filtrate used to antimicrobial activity. Antimicrobial activities were assayed by using well diffusion method (Gulve and Deshmukh, 2011) against the test organisms (*Granulicatella)* on sterile MH agar surface.

Results and Discussion

Nearly 13 Actinomycetes strains were isolated from soil sample. The colonies showing powdery growth were selected for further study. All strains of actinomycetes showed inhibition against the *S. auerus, Proteus* and *S. fecalis.* Actinomycetes strain D retain its antimicrobial activity against microorganism selected for secondary screening. The strains E lost inhibition potential during secondary screening.

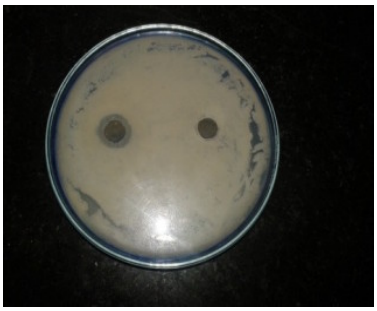
Table.1 Isolates showing antimicrobial activity

Test organism	Strain A	Strain B	Strain C	Strain D	Strain E	Strain F	Strain G	Strain H	Strain I	Strain J	Strain K	Strain L	Strain M
E. Coli	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve
E. Coli123	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve
E. Coli142	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Klebsiella	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
Klebsiella (MDR)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve
S. auerus	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Proteus	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
S. fecalis	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Granulicatella	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve
Pentoea	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve
Acinetobacter	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
Candida	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Providentia	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve

Note: -ve - Growth observed , +ve - Inhibition observed

Fig.1 Primary screening for strain D (Gaint plate technique) growth of test organism

Fig.2 Secondary screening for strains D and E (Well diffusion method) inhibition zone of inhibition against *Granulicatela*



The production of antibiotic where done by various researchers Jeffrey, L.S.H 2008 were isolate 62 Actinomycetes from Agricultural research center semongok, Sarawak and all showing antimicrobial activity against plant pathogens (Jeffrey *et al.*, 2007). During present work there is successful isolation of actinomycetes spp. from Ayurved college garden and Dr. BAMU Sub campus are Osmanabad and Tuljapur farm that showing antimicrobial activity against human pathogens. While Gayatri *et al.* (2011) isolates antimicrobial actinomycetes strain against the common human pathogen from marine habitat (Gayathri *et al.*, 2011).

The 13 srains were isolated from soil of the Ayurvedic college garden Osmanabad, Tuljapur farm district Osmanabad and Dr. Babasaheb Ambedkar Marathwada University Sub Campus, area Osmanabad, Mharashtra, India. All isolates were screened for antibiotic producing ability glycerolasparagine agar media. In primary screening, all isolates showed good ability for producing antimicrobial compound but in secondary screening only D strain showed retentions of inhibitory action.

Acknowledgement

The authors would like to thank guide, coguide and the head of department of microbiology and other members of microbiology department of Dr. B.A.M.U. Sub Campus Osmanabad.

References

Abebe Bizuye, Feleke Moges, Berhanu Andualem, 2013. Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town. *North West Ethiopia Asian Pac J Trop Dis.*, 3(5): 375–381.

Amit Pandey, Imran Ali, Kailash Singh Butola, Tanushri Chatterji, Vidyottma Singh, 2011. Isolation and characterization of actinomycetes from soil and evaluation of antimicrobial activities of actinomycetes against pathogen. *Int. J. Appl. Biol. Pharm. Technol.*, 2: 384–392.

Bhagabati Pandey, Prakash Ghimire, Vishwanath Prasad Agrawal, 2005. Studies on the antibacterial activity of the Actinomycetes isolated from the Khumbu Region of Nepal. *J. Culture Collect.*, 4: 29–35

El-khawaga, M.A., Megahed, M.M. 2012. Antibacterial and insecticidal Activity of actinomycetes isolated from sandy soil of (Cairo-Egypt) Egypt. *Acad. J. Biol. Sci.*, 4(1): 53–67.

Gayathri, A., Madhanraj, P., Panneerselvam, A. 2011. Diversity, antibacterial activity and molecular characterization of actinomycetes isolated from salt pan region of kodiakarai, Nagapattinam DT Asian. *J. Pharm. Tech.*, 1(3): 79–81.

Gulve, R.M., Deshmukh, A.M. 2011. Enzymatic activity of actinomycetes isolated from marine sediments. *Recent Res. Sci. Technol.*, 3(5): 80–83.

Gunasekaran Mohanraj, Thangavel Sekar, 2013. Isolation and screening of actinomycetes from marine sediments for their potential to produce antimicrobials. *JLBPR*, 2: 2250–3137.

Jeffrey, L.S.H., Sahilah, A.M., Son, R., Tosiah, S. 2007. Isolation and screening of actinomycetes from Malaysian soil for their enzymatic and antimicrobial activities. *J. Trop. Agric. Food Sci.*, 35(1):159– 164.