International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 706-712
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Antibiotic Production and Its Antibacterial Activity Against Clinical Pathogens Isolated from Wounds

[1]Raut Jyoti, [1]Sonune Yogeeta, [1]Warudkar Radhika and Chavan Neha*

PG Students and Assistant Professor*, Department of Biotechnology, Shivchhatrapati College, N-3, CIDCO, Aurangabad-431001, Maharashtra, India.

Received: 16 Jan 2019 / Accepted: 18 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: chavanneha08@gmail.com

Abstract

Actinomycetes are slow growing, Gram positive filamentous microorganisms. Actinomycetes are believed to be an excellent source of producing antibacterial substances with high commercial value. The Objective of this study is to determine antibacterial activity of antibiotic extracted from isolated Actinomycetes against pathogens of infected wounds and to characterize extracted antibiotic by FT-IR analysis. In this study, crude antibiotic was produced and extracted from Actinomycete i.e. *Streptomyces sp.* isolated from soil. Total Six bacterial cultures were isolated from wound infected patients. They were identified based on Biochemical and Cultural characterization according to Bergey's Manual of Systematic Bacteriology as *Micrococcus sp., Staphylococcus sp., Corynebacterium sp., Enterococcus sp., Escherichia coli, Proteus sp.* The Antibacterial activity of crude antibiotic was studied against wound isolates by Agar well diffusion method. The highest zone of inhibition i.e.24 mm was found against *Enterococcus sp.* at 800 µg/ml concentration of crude antibiotic and lowest zone of inhibition was observed against *Corynebacterium sp.*i.e.14 mm at 200 µg/ml. The Antibiotic extracted from *Streptomyces sp.* showed good antibacterial activity against Gram positive isolates compared to Gram negative bacterial isolates.

Keywords

Actinomycetes, Antibacterial activity, Antibiotic, wound pathogens.

*****

INTRODUCTION

Actinomycetes are slow growing, gram positive bacteria with high G+C ratio. They resemble to fungi because of their filamentous appearance and spore production property [19] and they resemble to bacteria because of presence of Peptidoglycan in their cell wall and possession of flagella [13]. Actinomycetes play variety of roles like degradation of recalcitrant Xenobiotic compounds, organic matter present in soil and waste release from agriculture field and urban community [8]. Most Actinomycetes species have capability to synthesize

biologically active secondary metabolites such as antibiotics, herbicides, antitumor, anticancer, pesticides, antiparasitic, immunomodulator, vitamins and enzyme inhibitors [14, 15]. 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. Actinomycetes are believed to be an excellent source of producing antibacterial substances with high commercial value. More than 70% of naturally occurring antibiotics are believed to be isolated from different genus of Actinomycetes [7]. Wound infections have been a problem in the field of surgery for a long time. Most bacteria live on our skin and other parts of the body with little potential for causing disease because of first line defense within the body. Surgical operation, trauma, burns, diseases, nutrition and other factors affect these defences. The skin barrier is disrupted by every skin incision, and microbial contamination is inevitable despite the best skin preparation. The control of wound infections has not completely eradicated the problem because of development of resistance. [10]

The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by Methicillin resistant *S. aureus* (MRSA) and Vancomycin resistant Enterobacter (VRE). Most bacteria have multiple routes of resistance to any drug and once resistant, can rapidly produce vast numbers of resistant progeny [11].

The present study focuses on production and characterization of antibiotic from isolated Actinomycetes and to determine its antibacterial activity against clinical pathogens which are isolated from wounds of human patients.

MATERIALS AND METHODS

I. Production and Extraction of antibiotic from isolated Actinomycetes: -

Streptomyces sp. isolated from soil was used for antibiotic production and extraction. Shake flask fermentation was carried out for antibiotic production. *Streptomyces sp.* was inoculated into 250 ml Erlenmeyer flasks containing 200 ml of fermentation medium (soluble starch 25 g, glucose10g, yeast extract 2 g, $CaCO_3$ 3 g, Trace salts solution 1ml,

distilled water 1 liter, pH 7.5±0.2 [Trace salt solution –$FeSO_4.7H_2O$ – 0.5 g; $CuSO_4.5H_2O$ – 0.5 g; $ZnSO_4.7H_2O$ – 0.5g; $MnCl_2.4H_2O$ – 0.5 g in 100 ml of distilled water]. The flasks were incubated on a rotary shaker (160 rpm) at 30 °C for 7-8 days, which is followed by centrifugation at 8000 rpm for 15 minutes. The clear supernatant used as an antibacterial substance.

Ethyl acetate was use for the extraction of the antibiotic from the culture supernatant. The solvent was added to the supernatant in 1:1 proportion. Solvent -supernatant mixture was shaken for 30 min in separating funnel. The solvent was separated from the broth by a separation funnel and concentrated using vacuum rotary evaporator. The concentrate of crude antibiotic was collected and stored at 4^0C. [3]

II. FTIR Analysis of Extracted antibiotics

Ethyl acetate extract of *Streptomyces sp.* was subjected to partial characterization using spectroscopic methods. The FT-IR spectrum of the compounds was recorded on SHIMADZU FT/IR Spectrophotometer from Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Band positions are reported in reciprocal centimeters (cm^{-1}). The spectra were scanned in the range 400 to 4000 cm^{-1}.

III. Isolation and Identification of Bacterial Clinical Pathogens from Wound infected patients: -

A) Sampling: -Six Samples were collected in sterile saline aseptically with the aid of sterile cotton swab sticks from infected wound of six different patients attending Mohalkar accidents, Aurangabad and MIT Hospital, Aurangabad.

B) Isolation of Clinical Pathogens: -

Isolation was done by Spread plate technique. In this method, all the Samples of wound were diluted in saline and spread on Nutrient agar plates. Plates were incubated at 37^0C for 24 hrs. After incubation different bacterial colonies were selected and purified by streaking on Nutrient agar media.

C) Identification of Clinical Pathogens: -

Six different bacterial colonies were isolated from wound infected patients. Identification of isolates was done on the basis of standard microbiological techniques involving Cultural, Morphological and Biochemical Characteristics according to Bergey's Manual of Systematic Bacteriology [16].

IV. Antibacterial Activity of extracted Antibiotic against the isolated Clinical Pathogens: -

Agar well diffusion method was used to determine the antimicrobial activity of extracted crude antibiotic against wound isolates. Four different

concentrations of crude antibiotic were prepared in DMSO (1%) ranging from 200 µg/ml- 800 µg/ml. All the wound isolates were activated in Nutrient broth for 24 hours. After incubation, 1 ml of each activated bacterial culture was mixed in soft agar and then poured on solidified nutrient agar plates. All the plates were allowed to solidify. In each plate,6 mm diameter wells were punched. Then different concentrations of crude antibiotic (20µl) were dispensed in separate wells. Ampicillin was used as a positive control and DMSO (1%) was used as a negative control. The plates were incubated at 37^0C for 24 hours. After incubation, the diameter of zone of inhibition around the wells were measured and recorded. [5]

RESULTS AND DISCUSSION

Antimicrobial resistance has increased drastically in recent years and it has rapidly become a leading public health concern [1].A large number of Actinomycetes have been isolated and screened from soil in the past several decades, accounting for 70-80% of relevant secondary metabolites available commercially [12].In this work, one Actinomycete i.e. *Streptomyces sp.*isolated from soil was used for antibiotic production (Fig.No.1).Extraction was carried out by solvent extraction method using ethyl acetate in separating funnel as shown in Fig.No.2.The extracted antibiotic was analysed for the presence of different functional groups by FTIR spectroscopy. Interpretation of FTIR spectrum was done according to Infrared Frequencies described by Hadi M.(2011), Augustine S.K.,(2005) and Mathur N. et al. (2015).The FTIR spectrum of Ethyl acetate extract of *Streptomyces sp.* exhibited strong absorption bands at 3323.35, 1639.49, 1242.16, 1043.49 and 1377.17 cm^{-1} which corresponds to hydroxyl group (O-H stretch), Double bonds (C=C), Aryl O-Stretch ,C-O bends and Amines respectively (Fig.No.4 and Table No.1)).The FTIR spectrum of Crude antibiotic extract showed the presence of range of functional moieties which are present in previously reported antibiotics of *Streptomyces* species. More or less similar trend was observed by Augustine S.K. et al. (2005), when they tested the FTIR spectrum of ethyl acetate extract of *S.albidoflavus* PU23 that exhibited absorption bands at 3296 and 1031.8 $cm-^1$, which indicated hydroxyl groups and absorption at 1639 cm-1 indicating double bonding. The results reveal importance and need of complete structural and in vitro pharmacological studies to uncover the industrial importance of these extracted antibiotic.

In this study, we collected six different samples from wound or skin infected patients (Fig.No.4) and isolated total six clinical bacterial isolates from these samples (Fig.No.5). For identification, the results of Cultural Characterization (Table No.2) and Biochemical characterization (Table No.3) of isolated clinical pathogens were compared with Bergey's Manual of systematic Bacteriology. Six isolates such as CP 1,2,3,4,5 & 6 were identified as *Micrococcus sp.*, *Staphylococcus aureus. Corynebacterium sp.*, *Enterococcus sp.*, *E. coli*, and *Proteus sp.* respectively. Similar isolates from wound infections were reported by Ali M. et al. The result of Hosimin K. et al. (2012) showed that the rate of isolation of Gram negative bacteria was more than Gram positive from wound; this is in contrast with our finding. In our finding, Gram Positive, *S.aureus* was predominantly found in nearly all collected wound samples. Verma V.C. et al., (2012) in their study reported isolation of different types of bacteria from pus which also revealed predominance of *staphylococcus aureus* followed by *Klebsiella sp., Pseudomonas sp., E. coli, Proteus sp.*

Actinomycetes have been recognized as source of several secondary metabolites like antibiotics and lytic enzyme. Which make them useful as an antagonistic agent against pathogen [2]. We have studied the antibacterial activity of crude antibiotic extract against isolated clinical pathogens. The crude antibiotic extract showed good antibacterial activity against all wound isolates except CP5 i.e. *E.coli* (Fig.No.6). The highest zone of inhibition (24 mm) was found against isolate CP4 i.e. *Enterococcus sp.* at concentration of 800 µg/ml and the lowest zone of inhibition is 14 mm found against CP3 *Corynebacterium sp.* at 200 µg/ml concentration of extracted antibiotic (Table No.4). We also observed that extracted antibiotic was more active against Gram positive bacteria than Gram negative. Similar result was also reported by L. Ashok Kumar, et al. (2012). The difference between the sensitivity of Gram negative and Gram positive bacteria is due to their cell wall morphology. Gram negative organisms are more sensitive to antimicrobial compounds because they do not have outer layer of lipopolysacchride and their cell wall is more permeable to these compounds [18]. Antibiotic extracted from Streptomyces sp. showed narrow spectrum activity as it was efficiently inhibited all Gram positive isolates while unable to inhibit growth of Gram negative bacterium i.e. *E. coli* with exception of *Proteus sp.* The result of the antibacterial activity was in contrast with that of Mathur N. et al, (2015) in which highest zones were observed against Gram negative i.e. *E. coli.* Mathur N. et al. (2015) also reported 18 mm zone of inhibition against *S. aureus*, which is less compared to zone of inhibition observed

against *S.aureus* in this work i.e.20 mm. The finding of this work indicates that crude extract of *Streptomyces sp.* may be alternative antimicrobial substance as a tool for controlling human skin infections.

Fig.No.1. Production of Antibiotic from *Streptomyces sp.*

Fig.No.2. Extraction of Antibiotic

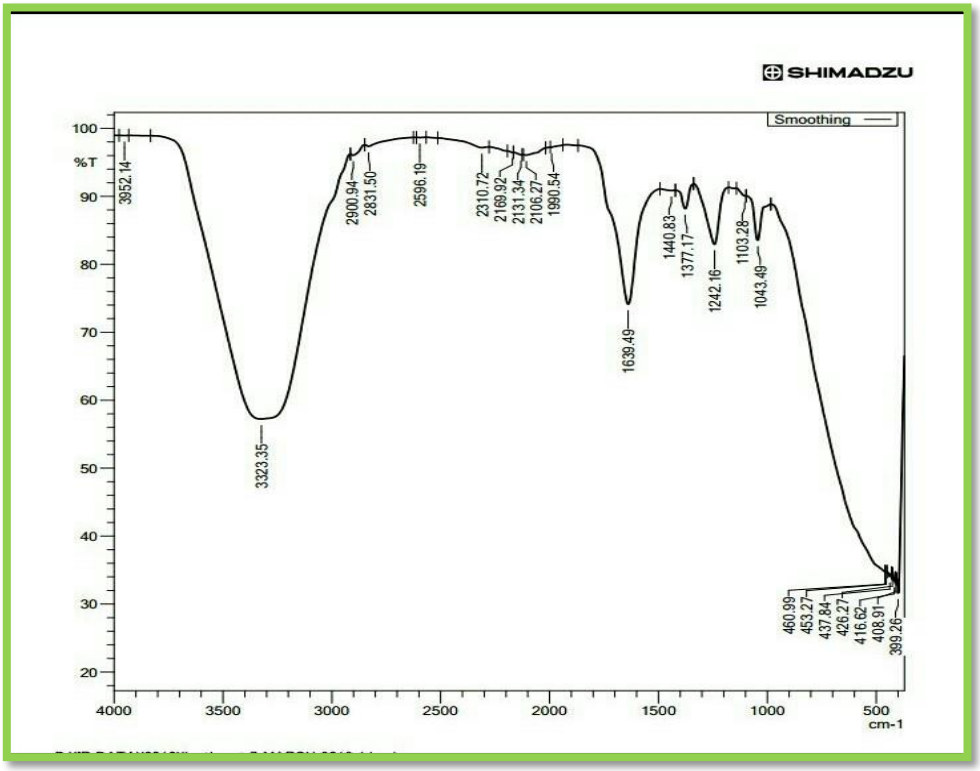

Fig.No.3. FT-IR spectrum of Ethyl acetate extract of *Streptomyces sp.*

Sr.No.	Frequency (Cm^{-1})	Functional Groups
1.	3323.35	Hydroxyl group (O-H stretch)
2.	1639.49	Double bonds, Alkenes (C=C)
3.	1242.16	Aryl O- Stretch
4.	1043.49	C-O bends
5.	1377.17	Amines (C-N stretch)

Table.No.1. FT-IR spectral data and functional group identification of Antibiotic extract

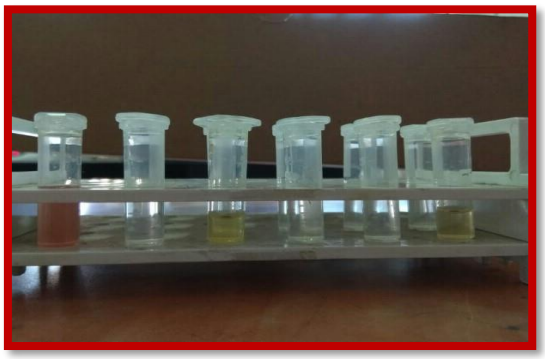

Fig.No.4. Collection of pus samples from wound

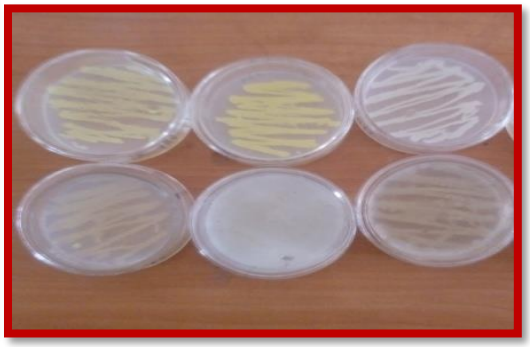

Fig.No.5. Isolation of Clinical Pathogens

Colony Characteristics	CP1	CP2	CP3	CP4	CP5	CP6
Size	1-2 mm	2mm	1 mm	1-2 mm	1 mm	2 mm
Shape	Round	Round	Round	Round	Round	Round
Color	Creamy yellow	Yellow	Off white	White	Off white	Grayish
Opacity	Opaque	Opaque	Translucent	Opaque	Translucent	Opaque
Elevation	Convex	Raised	Convex	Raised	elevated	Less convex
Margin	regular	Entire	Regular	Regular	Regular	Irregular
Surface	glistening	Shiny	Smooth	Smooth	smooth	Smooth
Consistency	Moist	Moist	Moist	Moist	Moist	Moist
Gram staining	Gram positive cocci (tetrad) arrangement	Gram positive cocci	Gram positive rod	Gram positive diplococci	Gram negative short rod	Gram negative short rod

Table.No.2. Colony characteristics of Isolated clinical pathogens (CP-Clinical Pathogen)

Biochemical tests	CP1	CP2	CP3	CP4	CP5	CP6
Catalase	Positive	Positive	Positive	Negative	Positive	Positive
Oxidase	Positive	Negative	Negative	Negative	Negative	Negative
Indole	Negative	Negative	Positive	Negative	Positive	Negative
MR	Positive	Positive	Negative	Negative	Positive	Negative
VP	Negative	Positive	Negative	Positive	Negative	Negative
Citrate	Negative	Positive	Negative	Negative	Negative	Positive
Glucose fermentation	Negative	Positive	Positive	Negative	Positive	Positive
Starch hydrolysis	Negative	Negative	Negative	Negative	Positive	Negative
Motility	Non motile	Non motile	Non motile	Non motile	Motile	Motile

Table.No.3. Results of Biochemical characterization of Clinical isolates

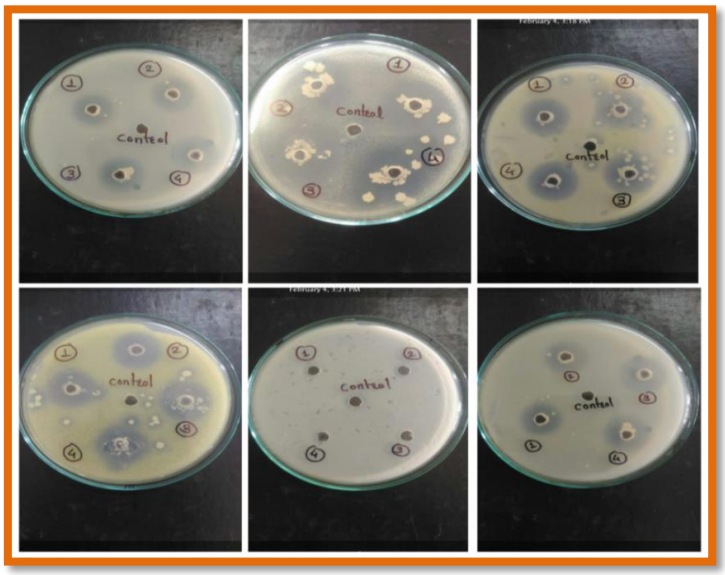

Fig.No.6. Antibacterial activity of crude antibiotic against clinical pathogens

Isolates	Diameter of Zone of Inhibition (mm)					
	200 µg/ml	400µg/ml	600µg/ml	800µg/ml	Negative control (1% DMSO)	Positive control(Ampicillin:200µg/ml)
CP1	17	18	20	20	-	34
CP2	16	17	18	20	-	30
CP3	14	15	16	17	-	28
CP4	20	21	22	24	-	34
CP5	-	-	-	-	-	26
CP6	18	20	21	22	-	32

Table.No.4. Diameter of zone of inhibition shown by extracted antibiotic against Clinical pathogens

ACKNOWLEDGEMENT

The Authors wish to acknowledge the Department of Biotechnology of Shivchhatrapati College, Aurangabad for their support and use of Department's laboratory facilities. We are thankful to the Head of Department, Principal of College and all teaching staff for their co-operation and support during the study. I would like to thank my guide for her counsel and guidance during this work. We would like to acknowledge Staff members of Mohalkar and MGM hospital, Aurangabad for providing samples.

REFERENCES

1. Ali M, Nas F.S, Yahaya A. and Ibrahim I.S., Characterization and determination of antibiotic sensitivity pattern of Bacteria from infected wound; *Annals of Micro. Inf. Diseases*; 1(1); 1-6.
2. Ashokkumar L., Balagurunathan R, Palanivel P. and Jegadeesh kumar D., Studies on antimicrobial activity of actinomycetes against MDR wound bacterial isolates, *Int.J.App.Bio.Pharma.Tech.*,2012,3(4),118-123.
3. Ashokkumar P and Karpagamp; Screening of antibiotic producing Actinomycetes from the sedimentsof undisturbed forest areas and its hyper activity after mutation, *Int. Adv.chem. Eng.Bio. Sci.Pharma. Tech;*2016,3(4).
4. Augustine SK, Bhavsar SP and Kapadnis BP. A Non-Polyene Antifungal Antibiotic from *Streptomyces Albidoflavus*PU 23. *Journal of Biosciences,*2005, *30*(2), 201-211.
5. Bauer A.W., Kirby W.M. M., Sherris J.C. and Turck M; Antibiotic susceptibility testing by a standardized disk method, Amer; *I. clin. Path.*1966, 45;493-496.
6. Hadi M. and OmidM.; Characterization of Streptomyces Isolates with UV, FTIR Spectroscopy and HPLC Analyses, *Bioimpacts*,2011,1(1): 47–52.
7. Henny R, Zakariya N.A.B and Das G K, Isolation and screening of antibiotic producing actinomycetes from soilsin Manong, Perak, Malaysia, *Der Pharmacia Lettre*,2016, 8 (15):226-229.

8. Heuer, H., Krsek, M., et al., Analysis of Actinomycete Communities by Specific Amplification of Genes Encoding 16S rRNA and Gel-Electrophoretic Separation in Denaturing Gradients, *Appl.Env. Micro.*, 1997,63(8): 3233–3241.
9. Hosimin K and Prabakaran G. studies on isolation and Characterization of some wound infection causing Bacteria; *Int. J. curr. Adv. Resear*. 2012,1(2); 26-31.
10. Howard, RJ., Ravitch, MM and Steichen, FM, Host against infections. Current problems in surgery. New English, *J. Medicine*.1980,12:1823-1830.
11. Livermore, DM. Bacterial resistance: origins, epidemiology, and impact. *Clin Infect Di*. 2003,36 (1), S11– S23.
12. Mathur N., Paliwal A., Sharma P. and Bhatnagarpradeep; Characterization of antimicrobial compounds from Streptomyces isolates; *J. chem... Pharma. Res*. 2015,7(4);1-10.
13. Mythili, B. and Das, M. P. A., Studies on antimicrobial activity of Streptomyces spp.isolates from tea plantation soil,*Res.J.Agri.Sci*.,2011,2(1);104-106.
14. Naikpatil, S. V. and Rathod, J. L. Antimicrobial and cytotoxic activity of actinomycetes from Karwar coast, west coast of India, *World J.Sci.Tech*, 2011,1(1): 07-10.
15. Oskay, M., Tamer, A. U. et al., Antibacterial activity of some actinomycetes isolated from farming soils of Turkey, Afri. J. Biotech.3(9);441-446,2004.
16. Sneath PHA, Mair NS, Sharpe ME, Holt JG; Bergey's Manual of Systematic Bacteriology, Vol.2, *Williams & Wilkins,*1986.
17. Verma V.C. and Chitra; Antibiotic sensitivity treatment for Gram negative bacteria isolated from Pus samples, *Int. J.Pharm.Bio.Sci.* 2012,2(3); 359-363.
18. Wahab A.,Subhan S.A.,Shumaila and Ali S.T. Isolation And Identification Of Actinomycetes Isolated From Karachi Soil And Screening Of Antimicrobial Compounds Int.J.Curr. R*esear,*2015*,* 7(2):2760-12765.
19. Waksman, S. A. On the classification of actinomycetes, *J. of Bacteriology*, 1940, 39(5): 549-558.