ISSN (O): 2349-5332
CODEN: IRJPHY



# INVITRO EVALUATION OF ANTIBACTERIAL EFFECT OF COLOURED ACTINOMYCETES

**Juluri Krishna Dutta Tejaswi*, Konda Ravi Kumar**

Department of Pharmaceutical Chemistry, Hindu College of Pharmacy, Amaravathi Road, Guntur (AP), India

Submitted on: 28.04.2020; Revised on: 12.05.2020; Accepted on: 20.05.2020

**ABSTRACT**

To discover novel secondary metabolites, our approach was to investigate unexplored regions of the world with the aim of isolating bioactive actinomycetes. The main objectives of the work was to select the antibiotic producing actinomycetes from cultures isolated from the marine environment and soil, to determine the antibacterial activity of the selected isolates and to determine the medium for antibiotic production by shake flask method. Isolation of colored actinomycetes from the samples with crowded plate technique and confirmed antibiotic production by giant colony technique. Fifteen colored strains were isolated and selected strains were checked for the antibiotic production with respect to different production medium. Both JKDSW5 and JKDSS7 showed antibacterial effect on Gram-negative bacteria.

**Key Words:** Isolation, Actinomycetes, Antibacterial activity, Rifampicin.

**Corresponding Author: J. K. D. Tejaswi**
**E-mail: v2kteja@gmail.com**
**Mobile: +91- 9966766337**

Indian Research Journal of Pharmacy and Science; 25(2020)2113-2120;
Journal Home Page: https://www.irjps.in
DOI: 10.21276/irjps.2020.7.2.2

## INTRODUCTION

The name actinomycetes were derived from Greek 'aktis'- a ray and 'mykas'- fungus. They were originally considered as intermediate group between Bacteria and Fungi but now were recognized as Prokaryotic organisms.[1] This was because, they had no nuclear membrane, sensitive to lysozyme for the most part and to the common antibacterial agents, there were similar to the type of bacterial flagella where these organelles were present and the type of cell wall resembles those of Bacteria.[2] Recent investigations focusing on marine derived actinomycetes isolates had yielded many new biologically active compounds and more importantly, a surprisingly much improved rate of discovery of novel compounds versus their terrestrial counterparts. Almost 80% of the world antibiotics were known to come from actinomycetes, mostly from the genera Streptomyces and Micromonospora.

## MORPHOLOGY

The majority of actinomycetes were free living saprophytic bacteria. They form hyphae with true branching as in the case of fungi and exhibit bacterial properties like lack of sterols and cell wall containing mucopeptides[3]. They were unicellular organisms which reproduce by fission or conidia or by special spores[4]. They produce a variety of spore types, which include the endospores long regarded as the typical spore structure of Eubacteriales[5]. Actinomycetes were the most economically and biotechnologically priceless prokaryotes[6].

## HABITAT

Actinomycetes are generally found in soil from gardens, fields, etc or terrestrial, sea water and sea bottom, fresh water basins, compromising lake and river waters and bottoms, food products including milk, atmosphere and geological formations etc.

## MARINE ACTINOMYCETES

Oceans cover more than 70% of Earth's surface and the organisms growing in marine environment were metabolically and physiologically different from terrestrial organisms. These marine actinomycetes produce different types of new secondary metabolites which possess biological activities and have the potential to be developed as therapeutic agents[7]. The actinomycetes were excellent in the production of bioactive secondary metabolites, a significant amount of effort had been focused on the successful isolation of novel actinomycetes from terrestrial source for drug screening for the past fifty years but recently, the rate of discovery of new compounds from terrestrial actinomycetes had decreased where as the rate of re-isolation of known compounds had increased[8]. The living conditions to which marine actinomycetes had to adapt during evolution range were extremely high pressures (with a maximum of 1100 atmospheres), anaerobic conditions at temperature just below ocean deep seafloor, at temperature over 100$^{o}$C near hydrothermal vents at the mid-ocean ridges and highly acidic conditions (pH as low as 2.8).

## ANTIBIOTICS

Antibiotics had completely transformed human approach to infectious diseases and contributed immensely to the welfare of human society. The original definition of an antibiotic was given as a chemical substance of microbial origin that possesses antibiotic powers[9]. They vary greatly in their morphology, physiology and biochemical activities including antibiotics and enzymes[10-14]. To estimate the actinomycetes species in a soil, pretreatment was done by adding calcium

carbonate, powdered chitin or pollen membranes which could increase Streptomyces colonies. It acts against most clinically relevant Gram positive bacteria and retains in vitro potency against isolates resistant to methicillin, vancomycin and linezolid.[15-17]

## MATERIALS AND METHOD

The materials used were Soya bean meal, Corn steep liquor, Glucose, Glycerol, Calcium carbonate, Sodium chloride, Meat extract, Tryptone, Yeast extract, Dextrose, Potato starch, Soluble starch, Casein hydrolysate, Ammonium sulphate, Peptone, Starch, Sodium nitrate, Di potassium hydrogen phosphate, Zinc sulphate and Agar.

## BIOLOGICAL EVALUATION

A total of 15 isolates were used for the screening of various activities. These isolates were isolated from different samples were collected into the sterile screw cap tubes with a sterile spatula and care was taken to see that the points of collection had widely varying characteristics as possible with regard to the organic matters, moisture content, particle size, colour of soil as shown in Tab.1.

**Tab.1: Samples collection with their Characteristics.**

| Sample | Collection |
|---|---|
| 1. | Sea sediment was collected a depth of 10 mts, Machlipatanum. The sample was muddy, blackish-brown in color. |
| 2. | Seawater was collected, at a depth of 10 mts Machlipatanum. |
| 3. | Seawater was collected, at a depth of 10 mts Suryalanka, Bapatla. |
| 4. | Sea sediment was collected near the shores Suryalanka, Bapatla. The sample was sandy, light brown in colour. |
| 5. | Soil was collected from college ground. |

## ISOLATION OF ACTINOMYCETES FROM SAMPLES

About 5 gm of sample was transferred to a sterile Erlenmeyer (E.M) flask containing 50 ml sterile water. The flasks were shaken on rotary shaker for 30 min for the detachment of the spore chains. The flasks were kept aside for 30 min to settle down the particulate matter. The clear supernatant was diluted into sterile water. These dilutions ($10^{-1}$ to $10^{-3}$ for samples) was used as inoculate. One ml of each of this dilution was pipette out into medium, plated into petridish (8 inch dia) and inoculated at $28^0$ C for 2-3 weeks. Sea water sample was also diluted from $10^{-1}$ to $10^{-3}$ for samples. All the media were sterilized by autoclaving. All the glass apparatus were sterilized by dry heat at $160^0$.C for 1 hr in hot air oven. Then incubated Petriplates were observed from 5 days onwards for 3 weeks. Rifampicin and Flucanazole were used to inhibit the bacterial growth respectively.

## STARCH CASEIN AGAR MEDIUM (SCA)

SCA medium was supplemented with Rifampicin-5µg/ml, cyclohexamide 50µg/ml to minimize bacterial contamination. After about one week to three weeks the plates were observed for colonies of actinomycetes. All such colonies were marked, identical colonies scored out and selected colonies were sub cultured into YEME slants and incubated at $28^0$C for one week as shown in Tab.2.

**Tab.2: Composition of Starch Casein Agar Medium.**

| Composition | Quantity |
|---|---|
| Soluble Starch | 10 gm |
| Casein | 0.3 gm |
| Potassium Nitrate | 2 gm |
| Sodium Chloride | 2 gm |
| $K_2HPO_4$ | 2 gm |
| $MgSO_4.7H_2O$ | 0.05 gm |
| Calcium Carbonate | 0.02 gm |
| $FeSO_4.7H_2O$ | 0.01 gm |
| Agar | 20 gm |
| Distilled Water | 1000 ml |
| pH | 7.2-7.5 |

**STUDY OF ANTIBIOTIC ACTIVITY**

The selected two isolates (i.e. JKDSW5, JKDSS7) were sub cultured onto YEME slants and incubated for about 7-10 days. The following production medium was used to test antibacterial activity. The following production medium was used to test antibacterial activity as shown in Tab.3 and Tab.4.

**Tab.3: Composition of Production Medium.**

| Composition | Quantity |
|---|---|
| Glucose | 1 gm |
| Yeast extract | 2gm |
| NaCl | 5 gm |
| $KNO_3$ | 3.8 gm |
| Half strength sea water | 1000 ml |
| pH | 7.4 |

5ml of sterile water was added to each YEME slant and organisms were scrapped with a sterile needle and transferred into 50 ml of production medium which was previously sterilized. The flasks were incubated at $30^0$C for 7 days on rotary shaker. After incubation, the broth was centrifuged and the supernatant broth was tested for extra cellular antibacterial activity as shown in Fig.1 and Fig.2.The following test organisms was used for screening.

**Gram positive bacteria*:*** Bacillus subtilis

**Gram negative bacteria:** Escherichia coli

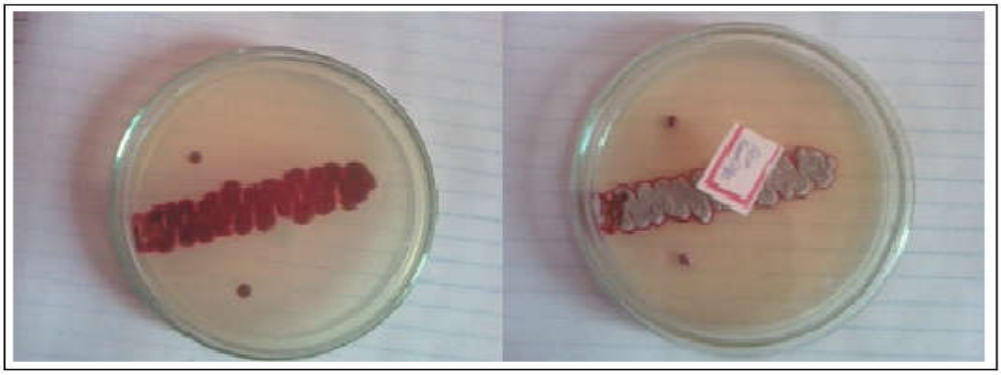

**Fig.1: JKDSW5 shows lower and upper surfaces characteristics**.

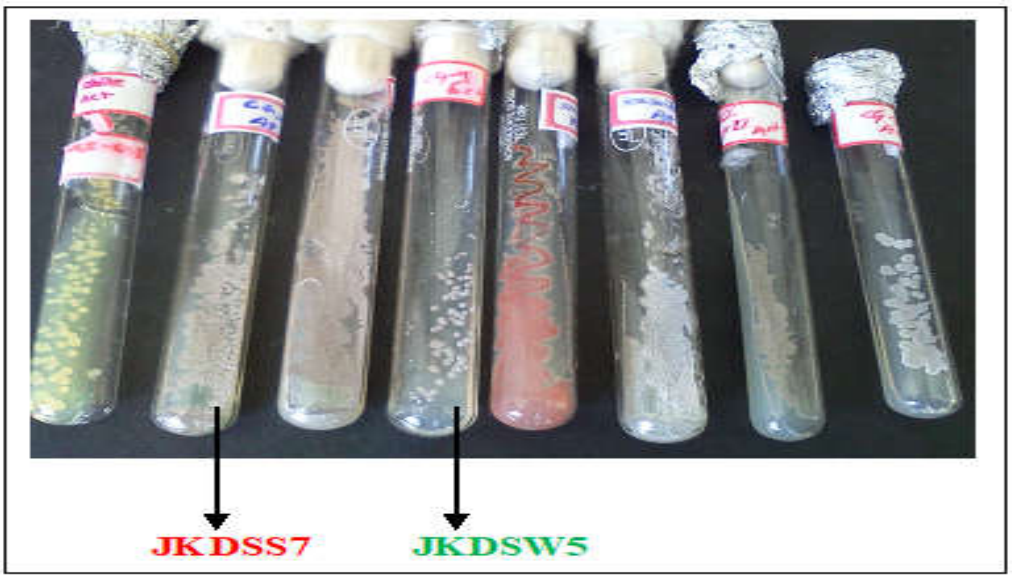


**Fig.2: Shows the various isolated from Marine and Soil Strains.**

**Tab.4: The following were used as Production Medium for Antibiotic.**

| **Medium 1 ($PM_1$)** | | **Medium 2 ($PM_2$)** | | **Medium 3 ($PM_3$)** | | **Medium 4 ($PM_4$)** | |
|---|---|---|---|---|---|---|---|
| Yeast Extract | **0.5%** | Soya Bean Meal | **1.5%** | Soya Bean Meal | **2.5%** | Glucose | **1.0%** |
| Dextrose | **1.0%** | Glucose | **1.5%** | Glucose | **2.5%** | Starch | **1.0%** |
| Starch | **2.0%** | Glycerol | **0.25%** | $NaNO_2$ | **0.4%** | Peptone | **0.75%** |
| Casein Hydrolysate | **0.5%** | NaCl | **0.5%** | $K_2HPO_4$ | **0.5%** | Meat Extract | **0.75%** |
| $CaCO_3$ | **0.4%** | $CaCO_3$ | **0.1%** | NaCl | **0.25%** | NaCl | **0.3%** |
| Distilled Water | **100 ml** | Distilled Water | **100 ml** | Distilled Water | **100ml** | Distilled Water | **100 ml** |
| pH | **7.0** | pH | **7.0** | $CaCO_3$ | **0.04%** | pH | **6.5** |
| - | - | - | - | ZnSO□ | **0.004%** | - | - |
| - | - | - | - | pH | **7.0** | - | - |

**CUP PLATE METHOD**

A 24 hr culture of freshly prepared test organism was seeded into the nutrient agar medium and was placed on to the Petri dishes. The cell free broth (50µl) samples along with the standards (Rifampicin of 1mg/ml conc.) were placed in the cups made at equidistant in the agar plate. Allowed to diffuse for 1 hr and incubated at 37ºC in a used as criterion for selection of isolate and production medium as shown in Tab.5, Fig.3 and Fig.4.

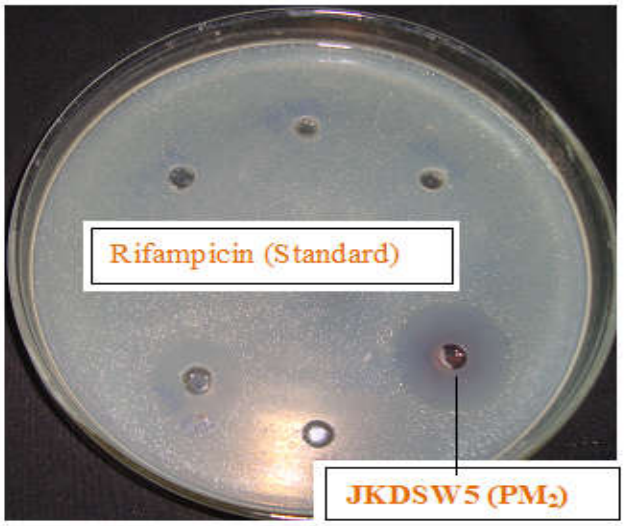


**Fig.3: Crude extract ($PM_2$) of JKDSW5 shows inhibition zones of Gram-Negative strain E.Coli.**

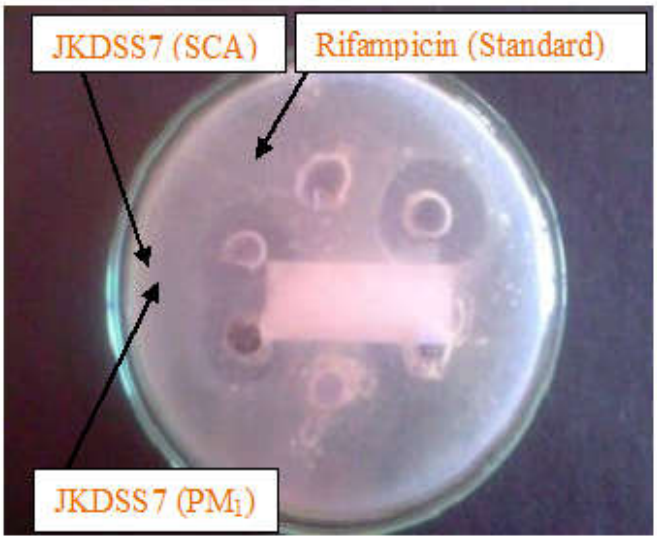


**Fig.4: Crude extract ($PM_1$) and SCA of JKDSW5 shows inhibition zones of Gram-Negative strain E.Coli.**

**Tab.5: Shows the inhibition zones of crude extracts and compared with reference standard.**

| S.No | Strain no | Inhibition zone diameter(mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Gram $^{+ve}$ bacteria (BS) | | | Standard | Gram $^{-ve}$ bacteria(EC) | | | Standard |
| | | $PM_1$ | $PM_2$ | $PM_3$ | | $PM_1$ | $PM_2$ | $PM_3$ | |
| 1. | JKDSW5 | NIL | NIL | NIL | 20 | NIL | 28 | NIL | 19 |
| 2. | JKDSS7 | NIL | NIL | NIL | 21 | 18 | NIL | NIL | 20 |

BS: *Bacillus subtlis* EC: *Escherichia coli*

Standard: Rifampicin 1mg/ml $PM_1$, $PM_2$ $PM_3$: Production medium

## RESULTS AND DISCUSSION

Various samples were collected from the different sources from the sample isolated the 15 strains, from these strains we were selected i.e. JKDSW5 and JKDSS7.These two strains were selected because they shows the antibacterial properties in crowded plate technique and gaint colony technique. To screen the anti microbial activities, we were selecting the two organisms they are gram positive Bacillus subtilis and gram negative E.Coli. The cup plate method showed no inhibition property of these strains were gram positive bacteria with respective production media $PM_1$, $PM_2$, $PM_3$ but we were observe inhibition zones of gram negative bacteria (E.Coli) with $PM_2$ of JKDSW5 and $PM_1$ of JKDSS7.The inhibition zones were compare with reference standard Rifampicin (1mg/ml) and observe JKDSW5 shows large inhibition zones when compared reference standard.

## CONCLUSION

The research work was done for the antibiotic producing actinomycetes from cultures isolated from the marine environment and soil and both the samples JKDSW5 and JKDSS7 showed anti bacterial activity only on gram negative bacteria with respective nutrient composition of production media.

## REFERENCES


1. Bhagabati Pandey. Studies on the Anti bacterial activity of the Actinomycetes isolated from Khumbu region of Nepal. Indian journal of microbiology, 2005; Pg: 118-121.
2. Buchanan R.E and Gibbons N.E. Bergey's manual of determinative bacteriology, 1974; Pg: 747.
3. Waksman S.A and Woodruff H.B. "Proceedings of the society for experimental biology and medicine". 1940; Pg: 45-609.
4. Kin S Lam. "Discovery of Novel Secondary Metabolites from Marine Actinomycetes". Current Opinion in Microbiology, 2000; 9(1); Pg: 245-251.
5. Fenical W and Baden D. Marine derived pharmaceuticals and related bioactive compounds. National Academic press, 1999; Pg: 71-86.
6. Grace Yim, Helena Huimi Wang, Julian Davies. "Truth about Antibiotics". International Journal of Medical Microbiology, 2006; 296(2-3); Pg: 163-170.
7. Bredholdt H, Galatenko O A, Engelhardt K, Terekhova L P and Zotchev S B. "Rare actinomycetes bacteria from the shallow water sediments of the Trondheim fjord, Norway: isolation, diversity and biological activity. Environmental Microbiology, 2007; 9(11); Pg: 2756-2764.
8. Hima Bindu M and Jetty. "Optimization of nutritional requirement for gentamycin production by Micromonospora echinospora". India Journal of Experimental Biology, 2006; 44(1); Pg: 842-848.
9. Naiyf S Alharbi. Novel Bioactive Molecules from Marine Actinomycetes. Biosciences Biotechnology Research Asia, 2016; 13(4); Pg: 1905-1927.
10. Shirling E B and Gottlieb D. Methods for characterization of Streptomyces species. International Journal of Systematic Bacteriology, 1966; 16(3); Pg: 312-340.
11. Jensen P R, Mincer T J, Williams P G and Fenical W. Marine Actinomycetes diversity and natural products discovery. Antonie Van Leeuwenhoek, 2005; 87(1); Pg: 43-48.
12. Naryana K J P and Vijayalakshmi M. "Optimization of antibacterial metabolites production by Streptomyces albidoflavus".

Research Journal of pharmacology, 2008; 2(1); Pg: 4-7.

13. Olano C, Mendez C and Salas J A. "Antitumor compounds from marine Actinomycetes". Marine Drugs, 2009; 7(2); Pg: 210-248.
14. Sujatha P, Bapiraju KV and Ramana T. "Studies on a new marine Streptomyces BT-408 producing polypeptide antibiotic SBR-22 effective methicillin resistant Staphylococcus aurous". Microbiological Research, 2005; 160(2); Pg: 119-126.
15. Sunga MJ, Teisan, Tsueng G, Macherla VR and Lam KS. "Seawater requirement for the production of lipoxazolidinones by marine actinomycetes strain NPS8920". Journal of Industrial Microbiology and Biotechnology, 2008; 35(7); Pg: 761-765.
16. Takahashi Y and S Omura. Isolation of Actinomycetes strains for screening of bioactive compounds. Journal of General Applied Microbiology, 2003; 49(3); Pg:141-154.
17. Vonithini G, Murugan M, Siva Kumar K and Sudha S, "Optimization of Protease production by an actinomycetes strains PS-18A isolated from an Eustraine shrimp pond". African Journal of Biotechnology, 2008; 7(8); Pg: 3225-3230.

CONFLICT OF INTEREST REPORTED: NIL ; SOURCE OF FUNDING: NONE