Original article

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# Antibacterial and Antifungal Property of Actinomycetes Isolates from Soil and Water of Nepal

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## ABSTRACT

**Background:** Human pathogens are rapidly acquiring resistance to antibiotics leading to treatment failure. We carried out this study to isolate and screen actinomycetes strains that have potential to kill bacterial and fungal pathogens.

**Methods:** In this descriptive study 288 soil and water samples were processed by standard microbiological techniques at Central Department of Microbiology,Tribhuvan University from 2013 to 2015. Screened actinomycetes were cultivated for bioactive metabolite production and minimum inhibitory concentration (MIC) of metabolites were determined against bacterial pathogens including multidrug resistant bacteria and fungi.

**Results:** One hundred twenty isolates having antimicrobial property were screened. Out of them, four most potent strains, *Nocardiopsis prasina, Streptomyces violarus, Streptomyces krainskii* and *Streptomyces tsusimaensis* were identified all having both antibacterial and anti-fungal property.Highest zone of inhibition (ZOI)was given by *N. prasina* against *Candida albicans*(41.33 ±1.15mm) and among bacteria, maximum ZOI was against *Acinetobacter baumannii*(31.33±3.05mm). MIC value of metabolite of *N.prasina* was 0.125mg/ml for *E.coli* and *C. albicans.* It was 2.5 mg/ml each for methicillin resistant *Staphylococcus aureus* (MRSA), *A. baumannii* and *Salmonella* Typhi and 0.625 mg/ml for *Bacillus Subtilis*.

**Conclusions:** Bioactive metabolite producing actinomycetes were recovered from soil and tested against human pathogenic bacteria and fungiand found to have antibacterial and antifungal property.

**Keywords:** Actinomycetes; bioactive metabolite; MIC; zone of inhibition.

## INTRODUCTION

Antimicrobial resistance creates a great threat for effective prevention and control of several diseases caused by bacteria, fungi, viruses and other parasites. Multidrug resistant *Klebsiella pneumoniae*, fluoroquinolone resistant *E.coli*, third generation cephalosporin resistant *Neisseria gonorrhoae*, methicillin resistant *Staphylococcus aureus* (MRSA) and Colistin resistant enterobacteriaceae and many other microbes have been reported from different parts of the globe.[1] Human pathogens such as carbapenem resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and enterobacteriaceae have been given top priority. Similarly, vancomycin resistant enterococci, MRSA, fluoroquinolone resistant *Salmonella*, *Campylobacter* and *Shigella* spp. along with many other multidrug resistant pathogens create problem so that new antibiotics should be developed to address resistance problem.[2] Antibiotics are bioactive secondary metabolites produced by bacteria, fungi and plants. Among diverse microbes actinomycetes are most capable candidates of producing antibiotics. Out of 22,500 biologically active compounds obtained from microbes, 45% are from actinomycetes, 38% from fungi and 17% from other bacteria.[3] Over 5000 antibiotics have been identified from the culture of Gram +ve, Gram -ve bacteria and fungi.[4] Among actinomycetes, various *Streptomyces* spp. account for more than 70% of total antibiotic production followed by other species.[5] Actinomycetes are Gram +ve, filamentous bacteria with high guanine+cytosine content of over 55% in their DNA.[6] Actinomycetes are natural inhabitant of soil, fresh water, marinewater, lakes and sediments and even found in extreme environment such as Himalayas and hot springs.[7] Hence, this study was conducted to isolate and screen potent antibiotic producing strains of actinomycetes against bacterial and fungal pathogens.

## METHODS

This study was carried out at Central Department of Microbiology, Tribhuvan University. In this study, 240 soil and 48 water samples were collected from different geographical locations of Nepal and actinomycetes

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were isolated by spread plate technique on starch casein agar.[8] Isolates were primarily screened for antimicrobial property against bacterial and fungal pathogens by perpendicular streak method on Mueller Hinton agar.[9] The strains showing antimicrobial property were cultivated under optimum conditions in starch casein broth at 30°C for 7 days at 150 rpm and bioactive metabolites were extracted in ethylacetate. The extracts were subjected to secondary screening against *Bacillus subtilis*, MRSA, *E.coli*ATCC25922, *Acinetobacter baumannii*(MDR), *Salmonella* Typhi(MDR) and *Candida albicans* by agar well diffusion method.[10] Minimum inhibitory concentration(MIC) of extracts were determined by tube dilution method.[11] Strains exhibiting both antibacterial and antifungal activities were characterized phenotypically on the basis of gram straining, sugar utilization test (Glucose, Mannitol, Sucrose, Fructose, Xylose etc.) substrate utilization test such as starch casein and gelatin, tolerance to different temperatures and sodium chloride concentration.[12] Molecular characterization was carried out by extracting DNA running PCR using universal primer and sequencing.[13]All the data generated were entered in excel file and SPSS version 20 and mean, standard deviation, frequency and percentage were calculated.

## RESULTS

A total of 288 soil and water samples from different geographical locations of Nepal were subjected to isolation of actinomycetes. Altogether 120 different actinomycetes showing antimicrobial properties were separated on the basis of pigmentation (Figure 1). Actinomycetes strains producing white and dirty white pigments were most predominant each 17%.

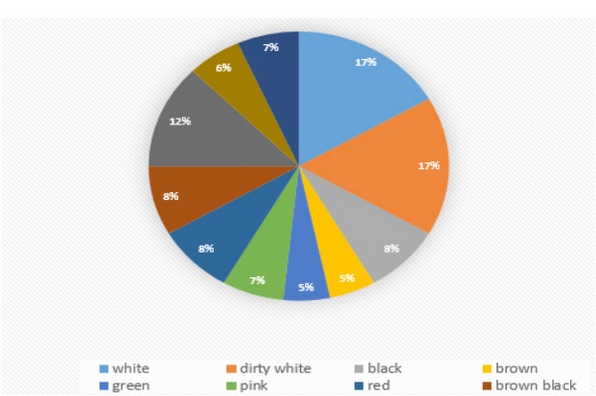


Figure 1: Types of actinomycetes on the basis of pigmentation

Among them 60 isolates were active against only Gram +ve bacteria, 44 isolates against only Gram -ve bacteria, 6 isolates showed activity against both Gram +ve and Gram -ve bacteria while 4 isolates exhibited both antifungal and antibacterial activity. Four most potent isolates were identified as *Nocardiopsis prasina*($A_3$), *Streptomyces violarus* ($D_2$), *Streptomyces krainskii* ($P_4$) and *Streptomyces tsusimaensis* ($J_1$) (Table 1).

**Table 1. Activity of bioactive actinomycetes against bacteria and fungi.**

| S.N. | Colony color | Active against | | | |
|---|---|---|---|---|---|
| | | Gram +ve only | Gram -ve only | Both Gram +ve and Gram -ve | Fungi and Bacteria |
| 1 | White | 8 | 8 | 2 | 2 |
| 2 | Dirty White | 8 | 8 | 3 | 1 |
| 3 | Black | 3 | 7 | 0 | 0 |
| 4 | Brown | 1 | 5 | 0 | 0 |
| 5 | Green | 4 | 2 | 0 | 0 |
| 6 | Pink | 6 | 2 | 0 | 0 |
| 7 | Red | 4 | 6 | 0 | 0 |
| 8 | Brown Black | 7 | 3 | 0 | 0 |
| 9 | Grey | 10 | 3 | 1 | 1 |
| 10 | Yellow | 7 | 0 | 0 | 0 |
| 11 | Purple | 2 | 0 | 0 | 0 |

Antimicrobial activity of bioactive compound extracted in ethyl acetate is shown in Table 2. Out of 4 potent isolates *N. prasina* showed highest antimicrobial activity against *Candida albicans* (41.33±1.15), *Salmonella* Typhi(24.33±2.08), *Acinetobacter baumannii* (31.33±3.05), MRSA (30.67±7.02) and *Bacillus subtilis*(24.67±2.08) while *S.krainskii* showed highest zone of inhibition against *E.coli* (26±0.00).

In MIC evaluation of bioactive compound, *Nocardiopsis prasina* showed lowest values against all test organisms 0.125mg/ml for *C. albicans* and *E.coli*, 2.5mg/ml for S. Typhi, *A. baumannii* and MRSA. For *Bacillus subtilis* all isolates gave same value 0.625mg/ml (Table 3).

**Table 2. Antimicrobial activity of extract against selected pathogens.**

| Strain | Zone of inhibition (mean±S.D.)mm | | | | | |
|---|---|---|---|---|---|---|
| | *Bacillus subtilis* | MRSA | *E.coli* ATCC 25922 | *Acinetobacter baumanii* | *Salmonella Typhi* | *Candida albicans* |
| A3 | 24.67±2.08 | 30.67±7.02 | 23.33±4.93 | 31.33±3.05 | 24.33±2.08 | 41.33±1.15 |
| D2 | 17.33±1.15 | 19.33±1.52 | 15.00±1.00 | 18.33±1.15 | 14.00±1.00 | 24.67±1.52 |
| PY | 24.33±0.57 | 31.33±3.21 | 26.00±0.00 | 28.00±2.00 | 19.00±1.00 | 29.33±0.57 |
| J1 | 14.33±2.08 | 23.33±2.08 | 24.67±0.57 | 21.67±1.52 | 17.33±0.57 | 28.67±1.52 |

**Table 3.MIC values of bioactive compounds from different strains.**

| Strains | MIC against(mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | *B. Subtilis* | MRSA | *E.coli* ATCC25922 | *A.baumannii*(MDR) | *S.*Typhi(MDR) | *C. albicans* |
| $A_3$ | 0.625 | 2.5 | 0.125 | 2.5 | 2.5 | 0.125 |
| $D_2$ | 0.625 | 5 | 2.5 | 5 | 5 | 2.5 |
| $P_4$ | 0.625 | 5 | 2.5 | 2.5 | 2.5 | 2.5 |
| $1(J_1)$ | 0.625 | 5 | 5 | 5 | 2.5 | 2.5 |

## DISCUSSION

In this study, we have isolated and screened bioactive compound producing actinomycetes strains. White and gray colored actinomycetes were predominant and most of them were active against Gram +ve bacteria. This result is similar with Mabrouk and Saleh( 2014) who reported dominance of white and gray actinomycetes with 64.3% active against Gram +ve bacteria.[9] Higher susceptibility of Gram +ve bacteria is due to lacking outer lipopolysaccharide which is impermeable to lipophilic compounds.[14] Results of our study is supported by Vengadesh et al., who found actinomycetes isolate $A_5$ was inhibitory to *Bacillus subtilis, E.coli, C.albicans and Aspergillus flavus*.[15] Our results are in agreement with Singh et al (2016) who observed high antibacterial activities of three actinomycetes strains against many test bacteria including MRSA, vancomycin resistant enterococci (VRE) and *Klebsiella pneumoniae*.[16] Results of this study showed that all four potent actinomycetes were active against bacteria and fungi with *N. prasina*as best candidate. MIC values of bioactive metabolite ranging from 0.125mg/ml to 5mg/ml. Similar to our findings, MIC value of metabolite of active actinomycetes as 1.25mg/ml for MRSA and other many bacteria. Similarly, MIC was 2.5mg/ml against VRE, *Shigella dysenteriae* and *Klebsella pneumoniae*.[17] In contrast, Satish and Kokati(2017) reported low MIC value of 1mg/ml for MDRSA.[18] MIC value is affected by many parameters including susceptibility of organisms, type of microorganism, concentration and type of bioactive metabolites, composition of cultural medium, incubation temperature and time.[15]

## CONCLUSIONS

Our study showed that soil and water of Nepal contained diverse actinomycetes strain that can inhibit the growth of some bacteria and *Candida albicans*. Among screened isolates *N. prasina* was found to be the most effective against test bacteria and fungi. Further studies regarding characterization of bioactive compound is essential.

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