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ISOLATION AND ANTIMICROBIAL AND DEGRADATIVE POTENTIAL OF ACTINOMYCETES

Padma Singh* and Vani Sharma

Department of Microbiology, Girls Campus Gurukul Kangri (Deemed University), Hardwar- 249 407, Uttarakhand, India

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Correspondence to Author:

Dr. Padma Singh

Head of the Department, Department of Microbiology, Girls Campus Gurukul Kangri (Deemed University), Hardwar- 249 407, Uttarakhand, India

E-mail: drpadmasingh06@gmail.com

ABSTRACT

Problem Statement: Do the soil Actinomycetes do have Antimicrobial and Petrol degradation potential? An intriguing question. Actinomycetes are continues to be a subject of study with reference to their Antimicrobial and degradative potential. However studies have been done is limited. Our object was to study its Antimicrobial activity in wide spectrum and to study its degradation potential on Petrol.

Approach: In this study we have isolated total 5 Actinomycetes from the Ganga river bed. All the isolates later purified and identified by various Morphological and Biochemical test. Here *Nocardia* was subjected to antimicrobial test against *Streptococcus, Mucor* and *Aspergillus* and it was also subjected to degradation test against Petrol.

Result: The 5 isolates are *Streptomyces, Micromonospora, Micromono sporangium* and 2 different strain of *Nocardia* (Na1 and Na2). The 2 strains of *Nocardia* are active against *Streptococcus* (Na1 29.6mm, Na2 26.6mm), *Mucor* (Na1 12.5mm, Na2 22.5mm) and *Aspergillus* (Na1 50%, Na2 60%). They also degrade Petrol very effectively, decrease in total organic carbon of the medium was observed during the degradation of petrol.

Conclusion: Our observation provides us with evidence that these agents can be used for the production of new antibiotics and as the agent to control the environment pollution.

INTRODUCTION: The Actinomycetes are Gram positive bacteria having high G + C content in their DNA. The name Actinomycetes was derived from Greek word 'aktis' (a ray) and 'mykes' (fungus) and given to these organisms from initial observation of their considered to be an intermediate group between bacteria and fungi but now considered as prokaryotic organisms, the majority of Actinomycetes are free living, saprophytic bacteria found widely distributed in soil, water and colonizing plants.

Actinomycetes population has been identified as one of the major group of the soil population [1], which may vary with the soil type. Actinomycetes are the most widely distributed group of microorganism in nature which primarily inhabit the soil [2], they have provided many important bioactive compound of high commercial value and continue to be routinely screened for new bioactive compounds. Almost 80% of the world antibiotics are known to come from Actinomycetes, mostly from genera *Sterptomyces* and *Micromonospora* [3].

Microbial antagonists are widely used for the biocontrol of fungal disease, Actinomycetes are the main source of antifungal hence highly used pharmacologically and commercially, these are the secondary metabolites of the Actinomycetes. The antagonistic activity of Actinomycetes to fungal pathogens is usually related to the production of antifungal compounds against *Fusarium oxysporum, Sclerotinia rolfsii* [4] (Lim et al 2000). Soil Actinomycetes particularly *Streptomyces* sp. enhance soil fertility and have antagonistic activity against wide range of soil born plant pathogens [5] (Aghighi et al 2004).

Action of Microorganism on Hydrocarbon: Many microorganism have the ability to utilize the hydrocarbon as the sole source of energy are widely distributed an enormous number and variety of organic compounds and were extremely important in the mineralization of organic matter. All the marine and fresh water ecosystems contain some oil degradation bacteria, no one species of microorganism, however is capable of degrading all the compounds of the given oil. The ability of a microorganism at a spill site is governed by the metabolic reactions. This ability is in turn governed by their genetic composition.

Enzymes produced by microorganism in the presence of carbon sources are responsible for attacking the hydrocarbon molecules, other enzymes are utilized to breakdown hydrocarbon further, lack of an appropriate enzyme either prevents attack or is a barrier to complete hydrocarbon degradation. At least two categories of enzymes are actively involved in biological degradation of polymers: extracellular and intracellular depolymerases during degradation.

Exoenzymes from microorganism breaks down the complex polymers yielding smaller molecules of short chains eg- oligomers, dimmers and monomers, that are smaller enough to pass the semipermeable outer bacterial membranes, and then to be utilized as carbon and energy sources. The process is called depolymerization, when the end products are CO_2, H_2O or CH_4 the degradation is called mineralization. When O_2 is available, aerobic microorganisms are mostly responsible for destruction of complex materials, with microbial biomass, CO_2 and H_2O as the final product. In the study, petrol were subjected to the biodegradation by ASTMD 5338 Standard.

MATERIALS AND METHOD:

Isolation and Identification of Actinomycetes: For Actinomycetes, soil sample were collected from the Ganga river bed. The sample collected are air dried aseptically, then one gram of above sample was suspended in 10 ml of sterile distilled water blanks, serial dilution was prepared by transferring 1ml of suspension to 9ml sterial distilled water blanks, 1ml suspension of 10^{-3}, 10^{-4}, 10^{-5} dilution was pour plate on Glycerol Yeast Extract Agar medium and incubated at 28^{o}C for 6 to 7 days. Actinomycetes was identified on the bases of various morphological and biochemical test such as Catalase, Starch Hydrolysis, Indole test, Methyl red test, Voges Proskauer test, Citrate utilization [6] (Aneja 2003).

Antimicrobial Assay: Its involve two methods;

1. Well Diffusion method [7] and;
2. Food Poison method [8].

1. **Well Diffusion method:** Sterile Glycerol Yeast Extract broth was taken and then it was inoculated with Actinomycetes culture, culture was kept in shaker at 28^{o}C for 48 hours. NAM was poured on sterile plates and allowed it to solidify after solidification 1ml of bacterial culture was transferred to a plate and spread it, well were prepared by cork borer. Now 0.8ml of Actinomycetes cultures were added and kept it for diffusion of solution at a room temperature for 30 min, plates were incubated at 37^{o}C and were examined and measure the zone of inhibition arounds wells.

Zone of inhibition = A-B

Here A= Zone of clearance; B = Cork borer diameter

2. **Food Poison method:** The antifungal activity of Actinomycetes was evaluated by using poisoned food technique; the fungi were inoculated on Potato Dextrose Agar (PDA) plates and incubated at 25^{o}C for 3 to 7 days to obtain young actively growing colonies of molds. 100ul of Actinomycetes extract was mixed with 15ml of cooled molten PDA medium and allow to solidify at room temperature for 30 min.

A mycelium disc 10mm diameter, cut out from periphery of 3 to7 days old culture was aseptically inoculated on agar plates containing the Actinomycetes extract. The inoculated plates were incubated at 25°C and colony diameter was measured and recorded after 7 days.

Percent mycelia growth inhibition was calculated as given below:

% Growth inhibition=

$$\frac{\text{Mean dia. of growth in control- Mean dia.of growth in test}}{\text{Mean dia. of growth in control}} \times 100$$

Biodegradation of Petrol: ASTM (American Society for testing and material) Process is used

1. Test flask with petrol was taken.
2. Blank flasks were cultured along with test flasks in duplicates, blank flask were devoid of any petrol.
3. Test flask was supplied with petrol respectively.
4. The amount of lubricants added was 0.0890-0.0815 mg in 2000 ml of medium, this amount of the petrol was applied to the culture flask by applying the lubricants on a clean grease free cover slip and introducing cover slip in the flasks.
5. inoculum was added in each blank and test flask, then the flasks were incubated at 25°C at 150 rpm on an incubation shaker for 15 days.
6. The evolved CO_2 was checked periodically in the absorber containing 100 ml of 0.0125 N $Ba(OH)_2$ each, the residual $Ba(OH)_2$ was estimated by titrating against 0.05 N HCl.

Co_2 evolved = N of HCL/2 x (T-b – T-s) x 44

- Here N of HCl is 0.05
- T-b is titration value of blank flask
- T-s is titration value of sample flask

RESULTS:

Isolation and Identification of Actinomycetes: Actinomycetes are isolated from the soil was calculated.

Table 1: CFU/ML COUNT

S. NO.	Dilution factor	CFU±SEM
1.	10^{-3}	10 ± 1.002
2.	10^{-4}	9 ± 5.014
3.	10^{-5}	7 ± 3.008

Later they are identified on the bases of morphological and biochemical tests.

Morphological characterization:

TABLE 2: MORPHOLOGICAL CHARACTERIZATION

Organism	Mycelium and nature of colony	Color of colony	Type of spores	Pigmentation	Gram stain
Streptomyces	Smooth, embedded in agar colony	White	Long chain of spores	Pale yellow	+ve
Micromonospora	Branched, mycelium, powdery colony	White to Gray	Monosporophore	-	+ve
Nocardia (Na1)	Powdery colony	Creamish white	Long chain of spores	Wine red	+ve
Nocardia (Na 2)	Powdery colony	Pinkish white	Long chain of spores	Pink	+ve
Microsporangium	Gummy colonies	Grayish white	Long chain of spores	-	+ve

Biochemical test:

TABLE 3: BIOCHEMICAL CHARACTERIZATION

S. NO.	Biochemical test	Na1	Na2
1.	Catalase	+	+
2.	Starch Hydrolysis	-	-
3.	Indole test	-	-
4.	Methyl red test	+	+
5.	Voges proskauer	-	-
6.	Citrate utilization	+	+

Here; + = Positive; –=Negative

Antimicrobial Activity of *Nocardia* (Na 1 and Na 2): The both culture were screened for antimicrobial properties against *Streptococcus, Staphylococcus, Mucor* and *Aspergillus*. Sensitivity and resistance was calculated by well diffusion and food poison method.

TABLE 4: ACTIVITY AGAINST *STREPTOCOCCUS*

S. NO.	Sample	Zone of inhibition (mm)
1.	Na 1	29.6
2.	Na 2	26.6
3.	Control positive BA	25
4.	Control negative D/W	–

Here: D/W = Distilled water; BA = CO – Trimoxazole

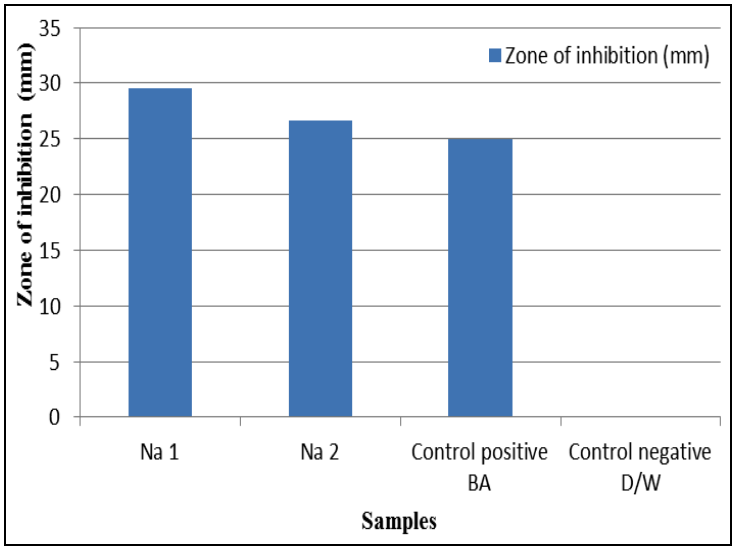

FIG. 1: ANTIBACTERIAL ACTIVITY CO-TRIMOXAZOLE WAS FOUND TO HAVE MAXIMUM ACTIVITY AGAINST *STREPTOCOCCUS* BY MULTIDISC DIFFUSION, USED AS POSITIVE CONTROL. *Staphylococcus* show complete resistance against both the strains.

TABLE 5: ACTIVITY AGAINST *MUCOR*

S. NO.	Sample	Zone of inhibition (mm)
1.	Na 1	12.5
2.	Na 2	22.5
3.	Control positive nystatin	22
4.	Control negative D/W	—

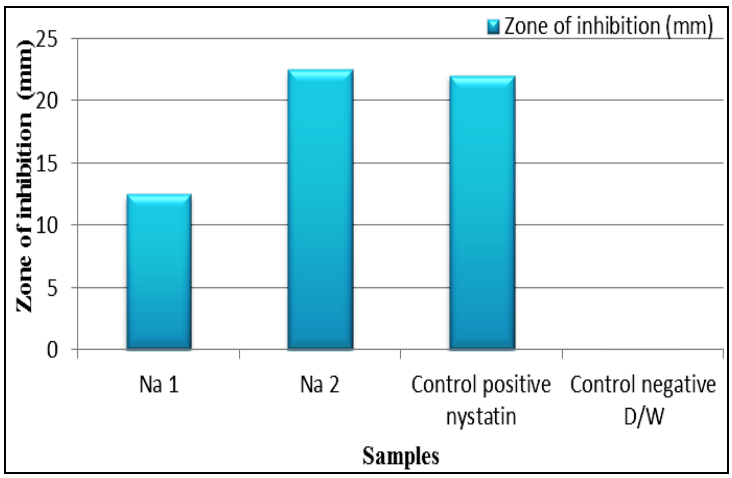

FIG. 2: ANTIFUNGAL ACTIVITY AGAINST *MUCOR*

TABLE 6: ACTIVITY AGAINST *ASPERGILLUS*

S. NO.	Sample	Radial growth (mm)	% of inhibition (mm)
1.	Na 1	15	50
2.	Na 2	16	60
3.	Control positive Nystatin	3	70
4.	Control negative D/W	–	–

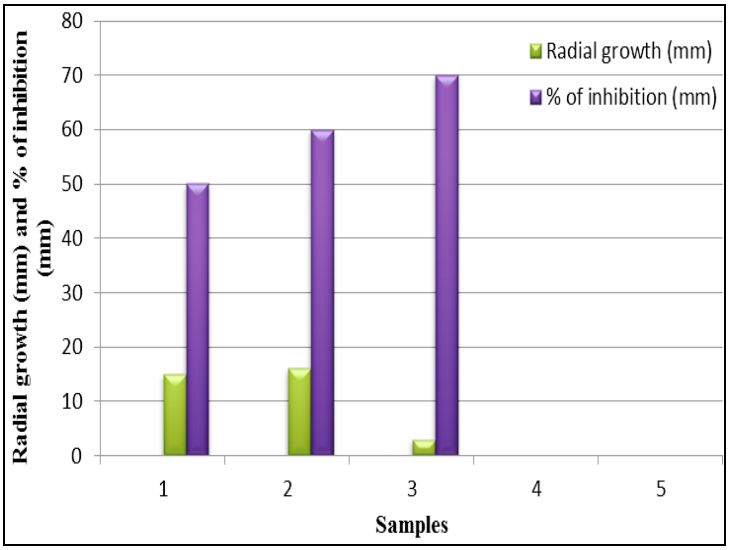

FIG. 3: ANTIFUNGAL ACTIVITY AGAINST *ASPERGILLUS*

Degradation activity of *Nocardia* (Na 1 and Na 2): Both cultures were now screened for degradation of petrol. The degradation of petrol was calculated by determining the CO_2 evolved in blank and test flasks during ASTM process.

TABLE 7: CO_2 EVOLUTION DURING DEGRADATION

S. NO.	DATE	CO_2 Evolved (mg) Na 1	CO_2 Evolved (mg) Na 2
1.	15.03.12	1	8.25
2.	16.03.12	4.73	1.54
3.	17.03.12	4.95	0.55
4.	19.03.12	3.3	0.44
5.	20.03.12	1.65	0.33

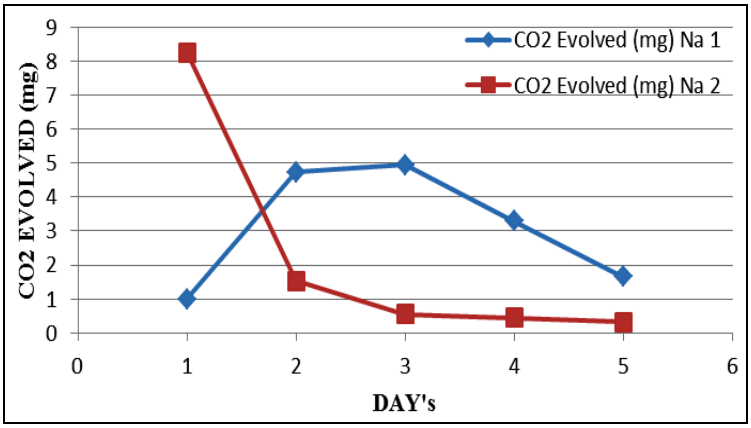

FIG. 4: DEGRADATION ACTIVITY AGAINST PETROL

DISCUSSION: The objective of our study was to explore the different properties of Actinomycetes in the novelty of our study was that, we have considered to study the antimicrobial and degradation property in single Actinomycetes. We observe that the soil sample collected from Ganga river bed have a huge variety of Actinomycetes ie contain $9x10^5$ cfu/gram of Actinomycetes (**Table 1**).

Actinomycetes colonies were observed as creamish, white, pinkish, gray, powdey, gummy as show in slants. A few biochemical tests are performed to identify these. Actinomycetes identified are 2 strains of *Nocardia* (Na 1 and Na 2) *Micromonospora, Streptomyces and Streptosporangium* (**Table 2 and 3**). Out of these we select *Nocardia* for our work.

Antibacterial potential was measured by the utilization of agar diffusion technique i.e. disc or well diffusion method, both the strains of *Nocardia* i.e. sample 1 and sample 2 shows the antibacterial potential against Gram positive (*Stereptococcus*) and show the zone of inhibition of 29.6 mm and 23.3 mm (**Table 4, Fig. 1**) respectively.

The both strain of *Nocardia* i.e. sample sample 1 and sample 2 was found to be inhibit the growth of *Mucor* by well diffusion method and zone of inhibition was come out to be 12.5mm and 22.5mm for Na1 and Na2 respectively (**Table 5 and Fig. 2**). *Aspergillus*, was also inhibited by food poision method and percentage of inhibition came out to be 50% and 60% for Na1 and Na2 respectively (**Table 6 and Fig. 3**).

On the other hand **Biodegradation** is a natural process where bacteria or other organisms alter and break-down of organic molecules into other substances such as fatty acids and carbon dioxide, which is not harmful to the environment. Many microorganisms having the ability to utilize hydrocarbons as sole source of energy are widely distributed in nature, no one species of microorganism, however is capable of degrading all the components of a given hydrocarbon. The hydrocarbons were found to be degraded more efficiently by a mixture of strains.

Recent technique for biodegradation, employed also in a project, makes use of population of microorganisms present in soil or sewage for degrading various petroleum products. This is because the ultimate destiny of various petroleum products used is either sewage or soil [9].

Two strains of actinomycetes were isolated from soil, measuring the CO_2 produced when petroleum is exposed to micro-organisms under control aerobic aquatic conditions helps to know the biodegradability of that product.

The sample of *Nocardia* that achieve a high degree of CO_2 evolution may be assumed to be easily biodegradable because CO_2 is evolved when micro-organisms are able to degrade hydrocarbon as their carbon source to gain energy, test substances that achieve a high degree of biodegradability in this test may be assumed as eco – friendly i.e. can be used in the environment without any harm. Various organisms have to be tested for their activity of biodegradation of petroleum such as thermophilic organism's [10].

In our study, we have found that both the strains show the capability to degrade petrol, but the strain Na 2 was found to be more efficient in degradation of petrol in comparision to Na 1 (**Table 7**).

CONCLUSION: We conclude that the selected Actinomycetes show's both antimicrobial and degradation potential. We infer that both strain can be used for the treatment of various bacterial and fungal infection. Further, they can also be used to control the environment pollution. Our finding we believe, substantiate and also provide ample scope for studies in this direction.

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