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# Studies on Isolation and Characterization of Antibiotic Producing Microorganisms from Industrial Waste Soil Sample

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**Abstract:** Antibiotics are one of the most important commercially exploited secondary metabolites produced by the bacteria and employed in a wide range. Most of the antibiotic producers used today are the soil microbes. Fungal strains and streptomyces members are extensively used in industrial antibiotic production. Bacteria are easy to isolate, culture, maintain and to improve their strains. Microbes are omni present and exist in a competitive environment. Bacillus species being the predominant soil bacteria because of their resistant endospore formation and production of vital antibiotics like bacitracin etc. are always found inhibiting the growth of the other organisms. In the present paper a soil bacterium with the antibiotic activity was screened and studied for morphological characters probably providing valuable information about the strain. The inhibitory activity of the organism was checked against some of the important opportunistic microbial flora and inoculated into an appropriate designed media depending on the bacterial requirements, and incubated for 48 hrs at $37^0$C. The produced compound was extracted by solvent extraction and assayed for its activity. Enhancement in the antibiotic production was studied under various parameters like temperature, pH, carbon source concentration, and sodium nitrate concentration, probably helping in the industrial production. The extracted substance was found effective against the gram positive endospore forming bacilli and gram positive cocci. Though a large list of antibiotics are known to be commercially available, the search for the most potential one is still on, and this work may provide some potential information on the antibiotic production and the control of microbial strains.

**Keywords:** Collection of soil samples, Isolation methods for bacterial and fungal spp, Kirby-Bauer method, Disk method, Dilutions, Bio-chemical characteristics and media preparation.

## INTRODUCTION

Antibiotics are the best known products of actinomycete. Over 5,000 antibiotics have been identified from the cultures of Gram-positive and Gram-negative organisms, and filamentous fungi, but only about 100 antibiotics have been commercially used to treat human, animal and plant diseases [1]. The genus, *Streptomycete,* is responsible for the formation of more than 60 % of known antibiotics while a further 15 % are made by a number of related Actinomycetes, Micromonospora, Actinomadura, Streptoverticillium and Thermoactinomycetes. Antibiotics, because of their industrial importance, are the best known products of actinomycetes [2-4]. The actinomycetes produce an enormous variety of bioactive molecules, e.g., antimicrobial compounds. One of the first antibiotics used is streptomycin produced by *Streptomycin griseus*.

On the whole, the last 55 years have seen the discovery of more than 12,000 antibiotics [4,5].

The actinomycetes yielded about 70 % of these, and the remaining 30 % are products of filamentous fungi and non-actinomycete bacteria [6,7]. Most of the bioactive compounds from actinomycete sort into several major structural classes such as amino glycosides (e.g., streptomycin and kanamycin), ansamycins (e.g., rifampin), anthracyclines (e.g., doxorubicin), b-lactam (cephalosporins), macrolides (e.g., erythromycin), and tetracycline. Besides, the use of microorganisms (fungi and bacteria); which serve as probiotics, antibiotics, enzymes and ensiling have constituted methods of enrichment of non-digestible feedstuffs and those imbued with anti-nutritive factors. In poultry, small quantities of tannins in the diet were found to have caused adverse effects, while the inclusion of additional proteins or amino acids into the diets may alleviate the effects of tannins [8]. Decrease methionine availability could increase the toxicity of cyanogenic glycosides; because methionine is involved in the detoxification of cyanide *via* methylation to thiocyanate [8]. Essential mineral elements, namely, magnesium, sodium, potassium, phospohorus and calcium are as important as amino acids and vitamins in maintenance of life, well-being and production [9]. Minerals are essential for regulating and building the living and aid in fighting depression [10-13]. Phosphorus and calcium are closely associated in metabolism, particularly in bone formation; phosphorus plays a critical role in the maintenance of acid-base balance, while calcium is essential. The main objective of the present investigation was to study the Isolation of antibiotic producing microbes (Bacterial and Fungal Isolates) ant their biochemical characterization from Industrial waste Soil as well as agricultural soil compost – Farmyard manure.

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## MATERIALS AND METHODS

Antimicrobial susceptibility testing methods were divided into types based on the principle applied in each system. They include (a) Diffusion; (b) Kerby-Bauer method/Disk method; (c) Dilution (Broth dilution, Agar dilution) ; (d) Minimum Inhibitory Concentration; (e) Diffusion and dilution; (f) E test method.

The efficacy of various antibiotic agents against a range of gram positive and gram negative microbial strains was assessed through measurement of zones of inhibition [14-17]. Dilution susceptibility testing methods were used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism [18-20]. Nutrient agar medium was prepared and sterilized follwed by proper pouring into Petri plates under aseptic conditions in a Laminar air flow. The Petri plates were kept for setting of medium for some time. The supernatant of production medium was collected and stored at 4 $^0$C. Antibiotic activity was assayed for the supernatants. Using Whatman paper discs were prepared and immersed in vials and let them to stand for 30 min. *E.coli* and Pseudomonas cultures were aspirated out. The cultures were swabbed on Petri plates followed by a proper placement of the antibiotic discs on the nutrient agar medium using [21, 22]. Further, these Petri plates were incubated overnight without inverting at optimum temperature of 37 $^0$C for 24 hours. Diffusion tests were used to determine the susceptibility of organisms isolated from soil samples of industrial waste areas.

### Isolation of Antibiotic Producers Soil Microbes

Thirty ml of normal saline was prepared and distributed equally into 3 test tubes, appropriate dilutions performed, and sterilized [23]. One gm of soil samplewas weighed and added into the stock test tube with 10ml of saline under sterile conditions., vortexed for 2 -3 mins, and allowed to stand for 2 min. One ml of sample from stock was transferred appropriately and shaken gently [24]. Finally, 0.5 ml of sample was collected from the dilution test tubes and spread plated. The plates were inverted and incubated over night at 37$^0$ C [25].

### Screening of the Soil

Eight actinomycetes were isolated from the soil samples [26]. They had pinpoint colonies with zone of inhibition. The presence of relatively large populations of actinomycetes in the soil samples of waste soil samples indicates that the source is an eminently suitable ecosystem. Pertinent details of the soil samples and the actinomycetes.

### Identification of Soil Bacterium from Secondary Cultures

Nutrient agar medium with 1% CaCO3 was prepared and sterilized [27]. The media was allowed to cool to 50-600c and poured into the sterilized Petri plates and allowed to solidify. Isolated colonies from slant culture was selected and taken carefully and streaked on the solidified medium in the Petri plates. The Petri plates were incubated at 37$^0$C overnight [28].

### Biochemical Characteristics of Antibiotic Producing Microbes

The isolate was biochemically characterized according to the Bergey's Manual of Determinative Bacteriology including indole production test, citrate utilization test, oxidase test, catalase test, starch hydrolysis, casein hydrolysis, and carbohydrate fermentation test performed to check the biochemical characteristics of producing strain [28]. Using an inoculating needle a colony was taken aseptically and put onto a clean slide and heat fixed. Crystal violet was added on the slide and allowed to stand for 60 sec and washed with distilled water. Gram's Iodine was added and left for 30 sec then the decolourizer was added appropriately till it became light violet [29]. Safranine was added on the slide and allowed to stand for 1 min, and the slide was washed with distilled water. Slide was air dried and observed under the light as well as electron microscope. Besides it was tested to determine whether the unknown bacterium was acid-fast.

For accomplishing antibiotic assay using well technique nutrient agar medium was prepared and sterilized and poured into Petri plates under aseptic conditions in a Laminar air flow. The Petri plates were kept for setting of medium for about 10 min and the wells were made at selected areas in Petri plates [29]. Thenafter the test organism's micrococcus and the bacillus strains were swabbed on the Petri plates with a sterile swab and left for 10 min. The crude extract obtained from centrifugation of production medium was aspirated and loaded into the wells with the capacity of 100 µl each. The Petri plates were incubated overnight with out inverting at optimum temperature of 37$^o$ C for 24 hours [30]. The comparison of inhibitory strains were monitored carefully and checked for the increasing concentrations of the antibiotic with effect to the increasing concentrations of the sodium nitrate [31].

## RESULTS AND DISCUSSION

The Streptomyces flora of 9 soil samples, collected from different locations in the Kanpur Industrial areas Kanpur, India, were screened for their potential as a source of antibiotics active against antibiotic resistant bacteria. All of the isolates were tested for their ability to produce inhibitory substances against seven test microorganisms (Fig. **1**). The results obtained showed similarties to those reported in the earlier studies [13, 32, 33] The test microorganisms included 3 Gram positive bacteria, 3 Gram negative bacteria and 1 yeast. Of them *S. aureus* and *S. maltophilia* were resistant to the widely used antibiotics (Table **1**). The isolates which exhibited antimicrobial activity against the test organisms have shown the resemblance to that reported in previous studies [34-37]. Six bacteria, including three Gram positive organisms were selected for this study. The morphological examination of these isolates, which were active on the test organisms, was observed to reflect that these belong to the *Streptomyces* genus. Gram positive forming bacilli were keenly observed. Strain was found contaminated. The enhancement in the potentiality of antibiotics i.e antimicrobial activity could be only due to exogenous incorporation of metal ions as well as complex compounds based on sodium, potassium, calcium, magnesium and phosphorus in different sets of experiments and cumulative effect of all together is reported Table **1**.

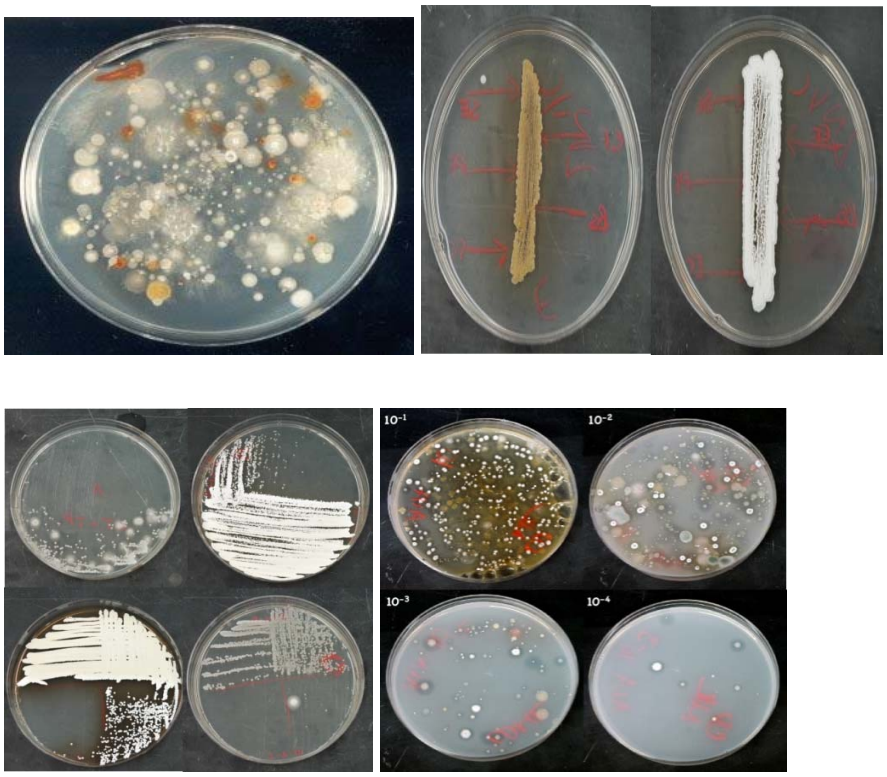


**Fig. (1).** Disk diffusion method or Kirby-Bauer antibiotic sensitivity test. Picture showing the growth of gram positive as well as gram negative bacterial and fungal isolates from soil sample from certain industrial areas.

**Table 1. Optimization of Potentiality Antibiotic. The Data were Summarized as the Cumulative Induction of Ions Such as: Sodium, Potassium, Calcium, Magnesium and Phosphorus**

| Concentration of $NaNO_3$ | Zone of Inhibition |
|---|---|
| 0.8 % | 1 cm |
| 1% | 1.1 cm |
| 1.2% | 1.4 cm |
| 1.4% | 0.9cm |

It is quite interesting to discuss the impact of exogenous supplementation of metal ions as well as complex compounds based on certain specific metal ions and minerals, on the growth of microbial organism (bacteria and fungi) as well as upscaling production of antibiotics from these organisms. In this reference the work accomplished in the preceding decade [8, 38] can be taken into consideration to justify the data obtained from the present study. Accordingly The levels of calcium and iron content of the mono-culture fermented mango kernel cake was observed to reflect added values to the mineral nutritional quality, as calcium being an essential factor for building living cells making up an animal body [38]. The quantity of magnesium recorded may still be adequate for animal nutrition since its help in keeping the muscles relaxed and used for the formation of strong bones and teeth in addition to controlling blood pressure and nerve transmitter [38]. Besides, the values of potassium, sodium, magnesium and other metal ions and minerals in most of the fermented mango kernel cakes may be adequate for animal feeding; thereby preventing the danger of muscular paralysis, mental disorientation and cardiac irregularities often associated with a fall in the level of potassium in the plasma. However, the levels of the individual essential amino acid of the fermented MKC suggested that there is still need for adequate supplementation of lysine and methionine, critically important for proper growth and development of poultry when the mono-culture fungal fermented MKC is to be used as chicken's feedstuff. Finally taken together the present study and the data reported in the previous reports [8, 38-40], the mono-culture of fungal and bacterial isolates applying towards production of antibiotics would certainly be quantitatively as well as qualititatively much more upgraded as a consequence of cumulative induction by certain specific metal ions and minerals. If those are present in the source soil, the isolation of microbes followed by standardization of antibiotic production should be carried out at the routine basis, but if it is contrary, the adequate supplementation of essential metal ions/minerals is quite mandatory for microbiologists to work along these notions and objectives in future.

## CONCLUSION

The soil microbial isolate isolated was showing antibiotic activity under normal growth conditions and was found inhibiting the gram positive bacilli and cocci. Crude extract of the microbial culture was proved showing the inhibition, which is further purified by the solvent extraction procedure .Sodium nitrate in increasing concentrations was found to have an enhancing effect on the bacterial antibiotic activity, but high concentrations that is more than 1.2 % was found detrimental. At 1.4 % the activity was found less inferring the effect of high concentrations of sodium nitrate in antibiotic production. Gram positive strains being more resistant because of their resistant spore formation were very hard to control. This work may provide an helpful information on the bacterial strain application in the industrial production of antibiotic that can easily control the gram positive strains. As the strain was a soil isolates its isolation, culturing and industrial exploitation aspects will be very easy to control. We suggest the strain improvement by the mutagenic agents and exogenous supplementation of certain specific metal ions and/or minerals to the microbial culture media probably being of great significance in view of enhancing the antibiotic activity. Besides, extraction and purification methods can be further employed for the pure antibiotic extraction.

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## CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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