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ISOLATION, IDENTIFICATION AND CHARACTERISATION OF ANTIBIOTIC PRODUCING BACTERIA FROM SOIL AT Dr C V RAMAN UNIVERSITY CAMPUS BILASPUR (C.G.)

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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. Bacteria are generally used and easily to isolate, culture, maintain and to improve their strains. Microbes are Omni present and exist in environment. Bacillus species, filamentous species, being the predominant soil bacteria because of their resistance endospore formation and production of vital antibiotics such as bacitracin etc. are always found inhibiting the growth of other microorganisms. *Actinomycetes* are aerobic, gram-positive bacteria that form branching filaments or hyphae and asexual spores. *Actinomycetes* produce most of the medically useful natural antibiotics. In this, project soil bacteria with the antibiotics activity have been screened and studied for morphological characters which can provide valuable information about the strain.

KEYWORDS: Antibiotics, *Actinomycetes,* morphological characters.

INTRODUCTION

Bacteria are microscopic living organisms, usually one celled that can be found everywhere. They can be dangerous, such as when they cause infection of beneficial as in the process of fermentation (such as in wine production) and that of decomposition.

Bacteria are single celled microbes. The cell structure is simpler that of other organisms as there is no nucleus or membrane bound organelles. Some of the bacteria have an extra circle of genetic material called as plasmid. The plasmid often contains genes that give the bacterium some advantage over other bacteria. For example- it may contains genes that makes the bacterium resistance to certain antibiotics.

Bacteria are classified into five groups according to their basis shapes like spherical (cocci), rod (bacilli), spiral (spirillia), comma (vibrio) and spirochetes. They can exist single cells in pairs, chains or clusters.

HISTORY OF BACTERIA

In 1676, Antonywen Leeuwenhoek first observed bacteria throughout microscope called them "animalcules". In 1838, the German Naturalist Christian Goltfried Ehrenberg called them "Bacteria" from the Greek bacteria meaning "little stick" and was shaped like rods. Antibiotics are a group of medicines that are used to treat infections caused by bacteria and certain parasites. They are sometime called antibacterial.

ANTIBIOTICS

Antibiotics can be taken by mouth as liquid, or capsules or they can be given by injection are in hospital because they have severe infection (palmubi 2001). Antibiotics are different types and used to treat different types of infection. The main type of antibiotics includes.

1. Penicillins – for example Penicillin v., Fluclorocillin and Amoxicillin.
2. Tetracycines – for example Tetracycline, Doxycycline andMinocycline.
3. Cephalosporins – for example Cafacior, Cefadroxil and Cephalexin.
4. Macrolides – for example Erythromycin, Azithromycin, and Clarithromycin.
5. Aminoglycosides – for example Gentamycin, Amikacine and Tobramycin.

Actinomycetes are gram positive bacteria with a high Guanine (G) and Cytosine (C) ratio in their DNA (<55 mol%), which are phylogentically related from the evidence of 16s ribosomal cataloguing and DNA rRNA pairing studies (Good fallow and Williams, 1983). The name "*Actinomycetes*" was derived from Greek "Atkins" (a ray) and "mikes" (fungus) and has features of both bacteria and fungi (Das *et al.*, 2008).

The *Actinomycetes* are a group of bacteria which possess many important and interesting features. They are of considerable value as producers of antibiotics and of other

therapeutically useful compounds. They exhibit a range of life cycles which are unique among the prokaryotes and appear to play a major role in the cycling of organic matter in the soil ecosystem (Veigoet al., 1983). In culture, agar plate *Actinomycetes* can be easily distinguished from true bacteria, unlike slimy distinct colonies of true bacteria which grow quickly, they appear slowly and show powdery consistency and also stick firmly to agar surface. *Actinomycetes* have gained prominence in recent year because of their potential for producing antibiotics (Kumar *et al*,. 2005).

Streptomycin, Gentamycin, Rifamycin some of the antibiotics which are in use presently and erythromycin is the product of *Actinomycetes.* The *Actinomycetes* are important in the field of pharmaceutical industries and also the agriculture. Jeffery et al., 2007 studied that, *Actinomycetes* isolated from Malaysian soil have the potential to inhibit the growth of several plant pathogens.

Actinomycetes are a specific group a bacteria, morphologically they resembleto fungi because of their elongated cells and branched into filaments or hyphae. During the composting mainly thermophile (adapted high temperature) and thermo-tolerate *Acinomycetes* are responsible for decomposition of the organic matter at elevated temperature (Jeffery et al., 2007).

Actinomycetes live predominantly aerobically i.e.; they need oxygen for their metabolism (oskay *et al*., 2004). Generally *Actinomycetes* grow on fresh substrates more slowly than other bacteria and fungi. During the composting process the *actinomycetes* degrade naturally substance such as chitin or cellulose. Some thermophile and thermo-tolerant *Actinomycetes* are found to be responsible for allergic symptoms in the respiratory tract.

MATERIAL AND METHODS

INSTRUMENTS

Autoclaves, (MAC), weighing machine (rove LECTRONICS), 20µl-1000µl micropipette, light microscope (Carlzeiss), water bath (MAC), Hot air oven, (Kasliwal brothers), horizontal laminar air flow(kasliwal brothers), refrigerators (whirlpool GNF 220).

Glass wares, plastic wares and consumable

Conical flasks, Beaker, Petri plates, Stirrer, Pipette, Test tube stand, Culture tubes, Slides, Reagent bottles, Cover slips, Forceps, Scalpels, Scissors, Wash bottle, Sucker, Gloves, Funnel, Burner tips, Tissue paper, Aluminum foil, Butter paper, Cotton, Test tube etc.

Chemicals

0.87% sodium saline, 1% CaCo3, 70% and 95% alcohol, crystal violet (0.1g), gram's iodine (0.18g), saffranine (0.2g), glycerine, 3% KOH, Malachite green (0.5g), carbolfuchsin stain, hydrochloride acid (conc. 3ml), methylene blue chloride (0.3g) beef extract (0.3%), zinc chloride (1g), potassium iodine (0.1g), powdered zinc metal, dil. HCL glucose (0.5%), yeast extract (0.5g), MgSO4 (0.02g), K2HPO4 (0.1g), NaCl (0.5g), methyl red (0.008g), tryptone (1g), potassium phosphate (0.5%), sodium citrate (0.2g), agar (1.5g), bromomethyl blue (0.08g), (NH4)H2PO4 (0.1g).

MEDIA USED

- Nutrient agar media
- Starch agar media

Collection of soil samples

The soil sample was collected in the month of January from top 4 cm soil profile where most of the microbial activity takes place and thus where most of the bacterial population concentrated. Soil sample (approximately 10g) was collected by using clean, dry, and sterile polybags along with sterile spatula, marking pen and other accessories. The site selection was done by taking care of the print where widely varying characteristics as possible with regards to the organic matter, moisture content and particle size and color of soil and to avoid contamination as far as possible. Samples were stored in the polybags and transported in laboratory where stored from the medicinal plant garden of CVRU campus of Dr C V Raman university Kota Bilaspur Chhattisgarh.

I. ISOLATION OF *ACTINOMYCETES*-

II. SCREENING

I. ISOLATION OF *ACTINOMYCETES*

PROCEDURE

30ml of normal saline is prepared and distributed into 6 test tubes were taking 10mlfor the stock, 9ml for 10ō1 and 10ō5 dilution and sterilized by autoclaving. 1 g of soil sample is weighed and added into the stock test tube with 10 ml of saline under sterile condition. The sample was over for 2-3 minutes and allowed to stand for 2minutes. 1ml of sample from stock is transferred into 10ō1 test tube and shaken properly. 1ml of sample from 10ō1 test tube was collected to transfer to the 10ō1 to 10ō5 test tub. 50ml of nutrient agar medium and starch casein agar medium is poured into the sterile plate and allowed to solidify 0.5 ml of the

sample was collected from the dilution test tube and spread on plate using a sterile glass spreader. The plates were incubated for 3-4 days at 37°C. After incubation powdery appeared colonies were incubated and maintained in the nutrient agar slant.

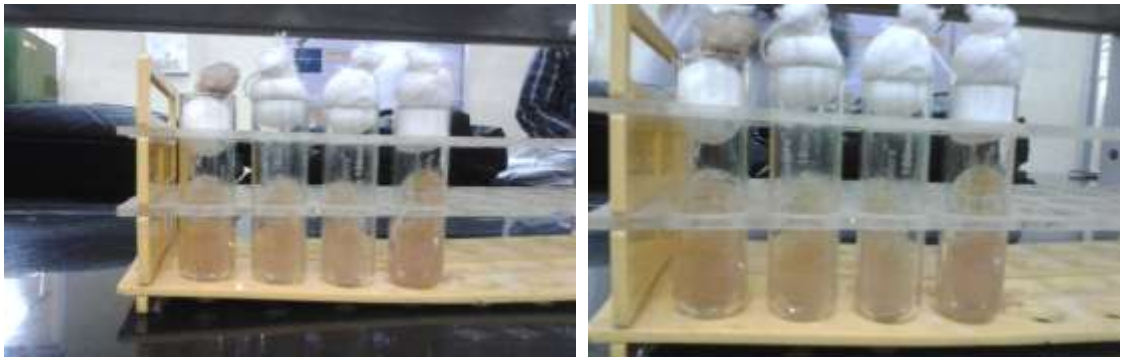

Fig 1: The Streaking done in Slant.

II. SCREENING

PROCEDURE

Starch casein agar medium with 1% CaCO3 was prepared and sterilized by autoclaved and the media was allowed to cool at 50-60°C and poured into sterilized petri plate and allowed to solidify. Isolated colony from slant were taken carefully with an inoculating loop and streaked on the solidified medium in the petri plates. The petri plates were incubated at 37°C for 1-2 days. After incubation morphological and biochemical characterization of isolates was done.

Fig:-2 The Screening done for the Isolated Colony

RESULT

Isolation of antibiotic producing bacteria (*Actinomycetes)*

Antibiotic producing bacteria was successfully isolated by the serial dilution method and streak plate technique. The serially diluted soil sample was for 3-4 days. The growth was seen in the plates of dilution 10-3 to 10-5. Plates having growth were sub cultured by streak in slant prepared by nutrient agar media and incubated at 1-2 days and pure colony was seen and preserved. The pure colony isolated was then screened in another plate having starch casein agar media 1% CaCO3.

Fig 3: Pictures demonstrating the colony growth after spreading in the plate.

The isolated strain was seen under inverted microscope, had the following characteristics

1. Gram staining	+ve
2. Acid fast staining	-ve
3. Endospore staining	+ve
4. Cell shape	Filamentous like cocci
5. Agar and culture characteristics	Broad, powdery and smooth
6. Oxidase test	+ve and –ve both
7. Catalase test	-ve
8. Indole production	+ve
9. Nitrate reductive test	-ve

10. Starch hydrolysis	+ve
11. Glucose fermentation	+ve
12. MR-VP Test	-ve
13. KOH Test	-ve
14. Citrate utilisation	+ve

CONCLUSION

Actinomycetes are the most economically and biotechnologically valuable prokaryotes that are used for the production of wide variety of enzymes, antibiotics and other bioactive compounds. There was not any scientific report on soil *Actinomycetes* producing antibiotic in the study areas. Therefore, isolation and screening of *Actinomycetes* from such areas in optimum condition may contribute the discovery of new antibiotics. Potent antibiotics from these *Actinomycetes* could contribute a lot to fight against antibiotic resistant pathogens. Further isolation identification and characterization are recommended to know the quality, novelty and commercial value of these antibiotics.

Actinomycetes are gram positive bacteria and are of considerable value as producers of antibiotics and other therapeutically useful compounds. The isolated *Actinomycetes* bacterial strains were gram positive, endospore forming, filamentous coccus shaped and forming colonies which were broad, powdery and smooth in texture. They showed oxidase and nitrate reductase enzyme activities. They utilized citrate and produced Indole along with starch hydrolysis and glucose fermentation. The present study suggests that soil surrounding the medicinal plants is highly rich in *Actinomycetes* bacterial species.

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