International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

In vitro Assessment of Antibiotic Efficacy of Streptomycin Isolated from *S. griseus* Against Selected Bacteria

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Abstract: ***S. griseus isolated from soil samples was screened for production of streptomycin by plate assay. Identification of streptomycin antibiotic was done by paper chromatography using selected strains of bacteria by measuring the zone of inhibition. Among all the bacterial strains studied, the highest zone of inhibition was measured against E. coli whereas lowest was measured against S. aureus after 24 hrs in nutrient agar media.***

Keywords: *S. griseus, E. coli, S. aureus,* Streptomycin, Paper chromatography.

1. Introduction

Actinomycetes are found in fresh water, seawater, and cold and warm-blooded animals and composts. Actinomycetes produce over 6,000 chemically different antibiotics of microbial origin and continue to be an excellent source of novel compounds. Many of these natural products are commercially important medicinal compounds with a variety of therapeutic uses **(Butler *et al.*, 2002).** Actinomycetes are now commonly referred to as actinobacteria. Actinobacteria are neat because they tend to produce cool secondary metabolites. Many of which have been successfully isolated and turned into useful drugs and other organic chemicals. The genus Streptomyces is a particularly fruitful source of these compounds, a number of which have been developed as antifungals, antibiotics (antibacterials) and chemotherapeutic (anticancer) drugs. Actenomycetes, mainly Streptomyces species, produce tetracyclines, amino glycosides (streptomycin and its relatives), macrolides (erythromycin and its relatives), chloramphenicol, ivermectin, rifamycins and most other clinically useful antibiotics that are not beta-lactams. **(Raja and Prabakarana, 2011).**

Streptomyces is the largest genus of actinobacteria and the genus of the family streptomycetaceae **(Kampfer, 2006).** Over 500 species of Streptomyces bacteria have been described by **Euzeby (2008**). As with the other actinobacteria, streptomycetes are gram-positive and have genomes with high GC-content **(Madigan and Martinko, 2005)**, which are found predominantly in soil and decaying vegetation. Most Streptomycetes produce spores and are noted for their distinct "earthy" odor which results from production of a volatile metabolite, geosmin. Streptomycetes are characterized by a complex secondary metabolism. They produce over two-thirds of the clinically useful antibiotics of natural origin; e.g., neomycin **(Kieser *et al.*, 2000).** Actinomycetes are mostly distributed wide range of environment like terrestrial and marine. The presence of extreme environment especially at cryophilic region also reported by **Raja *et al.* (2010b).**

Obviously various actinomycetes, first of all the Streptomyces species and filamentous fungi are the most producers in respect of numbers, versatility and diversity of structures of the produced metabolites. The significance and frequency of these main types of microbes as producers of bioactive metabolites had varied significantly during the last decades. In the beginning of the antibiotic era, the fungal (*Penicillin, Griseofulvin*) and bacterial (*Gramicidin*) species were in the foreground of the interest, but after the discovery of streptomycin and later chloramphenicol, tetracyclines and macrolides the attention turned to the *Streptomyces* species. In the fifties and sixties, the majority (70%) of antibiotics were discovered from these species. In the next two decades the significance of the non- Streptomyces actinomycetales species (rare actinos) were increased, up to a 25-30% share of all antibiotics. From the early nineties the number of bioactive compounds isolated from various filamentous and other microscopic and higher fungal species had continuously increased up to more than 50% by the turn of the millennium **(Kieser *et al.*, 2000).** Actinomycetes are Gram-positive bacteria frequently filamentous and sporulating with DNA rich in guanine and cytosine from 57-75 %. Some of their secondary metabolites have employed as useful microbial compounds **(Prescott *et al.*, 2002).** Example- streptomycin from *S. griseus* for treatment of tuberculosis caused by *M. tuberculosis* and the immunosuppressant drug, tacrolimus (FK506) produced by *S. tsukubaensis*. About 100 genera of actinomycetes exist in soil **(Yokota, 1997)** but none of them are able to produce streptomycin. The objective of the study is to evaluate

antibacterial efficacy of streptomycin secreted from *S. aureus* against selected bacteria.

2. Materials and Methods

Soil samples collected by sterile method from various locations nearby Mallathahalli, Bangalore, were air-dried under room temperature for about 5 days before isolation of microorganism. For this, 0.5 gm of soil samples was suspended in 9.5 ml of sterile distilled water and 0.1 ml of this was spread on four Petri plates with nutrient agar medium using 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} fold dilution, respectively, which were then incubated for bacteria, incubation was carried out at 37˚C±1˚C for 24 hrs whereas for actinomycetes**,** incubation was carried out at 28±1˚C for 3 days using starch casein nitrate agar media. The isolated bacteria were characterized on the basis of morphological and cultural characters by inoculating the selected strain onto nutrient agar media and starch casein nitrate agar media. Antimicrobial activities of isolates were tested preliminarily by cross streak method of **Lemos *et al.* (1985).**

2.1 Streptomycin production by Streptomyces griseus

Inoculum was prepared in starch casein nitrate broth containing soluble starch - 10, potassium phosphate dibasic - 2, potassium nitrate - 2, sodium chloride -2, casein -0.3, magnesium sulphate (7 hydrated) - 0.05, calcium carbonate - 0.02 and ferrous sulphate (7 hydrated) - 0.01 g/l ; inoculated *S. griseus*. The broth was incubated at 28˚C±1^{o}C for 7 days in an orbital shaker at 150 rpm for streptomycin production. On 8th days, production media was centrifuged at 6000 rpm for 10 minutes and supernatant was taken. Added 2 gm of activated charcoal followed by shaking the contents for 10 minutes. The content was filtered using Whatman no. 1 filter paper. The antibiotic was eluted from charcoal by spraying 100ml of methanol: HCl (9:1). The content was filtered and charcoal was removed after neutralizing the solution (pH-7) with NaOH. Equal volume of ethyl acetate was added and allowed to precipitate out followed by its centrifugation at 1000 rpm for 10 minutes and drying the organic layer in desiccator. Finally, eluted antibiotic was dissolved in phosphate buffer (0.25M and pH 7.2).

2.2 Identification of streptomycin

Identification of streptomycin produced was done by paper chromatography **(Snell *et al.*, 1956).** Ascending chromatograms on Whatman No.1 were developed containing 1 cm of solvent mixture (Acetone: Acetic acid: Water, 50:3:47). Samples were spotted 2 cm above the base of the paper, and dried thoroughly before placing in the solvent. After the solvent had migrated to the top of the paper (usually 12 cm) the chromatograms were air dried and exposed to steam to ensure adequate removal of acetic acid.

2.3 Measurement of zone of inhibition by agar diffusion method

Agar well diffusion method was used to check the cultures for the production of antimicrobial metabolites by **Sen *et al.* (1995).** 24hrs fresh cultures were diluted with pre-sterilized normal saline and were spread by spreader on solidified nutrient agar media. Wells were prepared over the nutrient agar plates. About 50µl cell free supernatant of streptomycin antibiotic was added in the wells and the plates were incubated at 37ºC for 24hrs. After 24 hrs, the zones of inhibition were recorded.

2.4 Statistical analysis

21 Petri plates were measured for statistical analysis from which 3 Petri plates for each culture were taken as triplicate. All data from this experiment were calculated as mean±SD.
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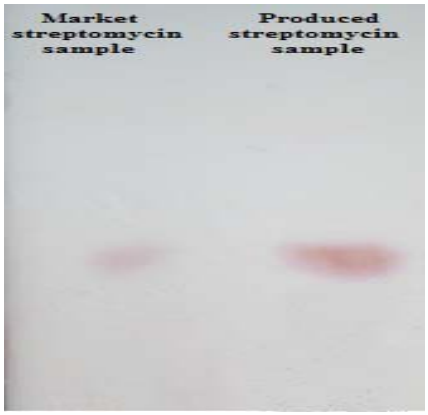
3. Results and Discussion

Mixed culture was observed in the nutrient agar media plate of seven strains of bacteria (*B. subtilis, B. megaterium, B. macerans, B. brevis, M. luteus, S. aureus* and *E. coli* respectively) and one Actenomycetes (*S. griseus*) were isolated from soil samples by starch casein nitrate agar selective media. The microorganisms isolated were identified based on their morphological, culture characterization and biochemical tests. On paper chromatography, the R_f value of streptomycin was found 0.5 conforming the compound as streptomycin.



(a)

(b)

Figure 1: (a) Paper chromatography of produced streptomycin sample showing R_f value compare with marketed antibiotic sample **(b)** Zone of inhibition of streptomycin against *E. coli* and *S. aureus*

The produced antibiotic was checked for antibacterial activity against 7 selected pathogens. Among all the bacterial strains studied, the highest zone of inhibition (24.53 ± 0.80 mm) was measured against *E. coli* whereas lowest (11.51 ± 0.6 mm) was measured against *S. aureus* after 24 hrs in nutrient agar media (Table 1, Figure 1) followed by *B. subtilis* (18.10 ± 0.82), *B. megaterium* (19.00 ± 1.14), *B. macerans* (20.43 ± 2.46), *B. brevis* (14.66 ± 1.50) and *M. luteus* (16.00 ± 5.30) respectively, for **s**treptomycin production from *S. griseus*. Streptomycin is a bactericidal antibiotic **(Sharma *et al*., 2007).** It is a protein synthesis inhibitor which binds to small 16S rRNA of the 30S subunit of the bacterial ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit. This leads to codon misreading, eventual inhibition of protein synthesis and ultimately death of microbial cells through a mechanism, which is still not very clear **(Voet and Voet, 2004).**

Table 1: Diameter of zone of inhibition against isolated strains*

Test microorganisms	*Diameter of zone of inhibition (mm)*
B. subtilis	18.10±0.82
B. megaterium	19.00±1.14
B. macerans	20.43±2.46
B. brevis	14.66±1.50
M. luteus	16.00±5.30
S. aureus	11.51±0.69
E. coli	24.53±0.80

*Diameter of zone of inhibition of cultures were measured in above table as mean±SD

4. Conclusion

In the present study, streptomycin was found to be most effective against *E. coli* and moderately effective against *B. macerans, B. megaterium, B. subtilis* and *M. luteius* and least against *B. brevis* and *S. aureus.*

5. Acknowledgement

The authors are thankful to Best Biotek Research Labs Pvt. Ltd., Bangalore for kind help and support to carry out the piece of work.

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