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Optimization of Medium for the Production of Streptomycin By *Streptomyces Griseus*

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ABSTRACT: *The present investigation was made to find out the optimal media for the growth of the Streptomyces griseus bacteria which is more useful for the production of Streptomycin. The soil sample was collected from the Jayanagar 4th block from Shalini park Bangalore. A specific media Starch Casine Agar (SCA) was used for the isolation and culturing of the bacterial strain. Characterizations of these strains were also studied by visual observation of colony, microscopic observation and biochemical tests identified the specific bacteria namely Streptomyces griseus. Antimicrobial activity of isolated bacteria was performed against E.coli bacteria. Estimation of Streptomycin sample was done with the help of HPLC. The isolated sample contained 80% of the Streptomycin per 100ml. Optimization of medium for the production of Streptomycin was done by on the basis of pH, Time, Carbon Source, Nitrogen source. Streptomyces griseus showed maximum growth at pH value of 9, incubation time of more than 72 hours, maximum growth in the medium having glycine as nitrogen source, and maximum growth in the medium which contain rice bran as a carbon source.*

KEYWORDS: *Bacterial isolation, characterization, antimicrobial activity, estimation of streptomycin by HPLC, Optimization of media.*

I. INTRODUCTION

Antibiotics are the antimicrobial agents, which are produced by some micro-organisms to inhibit or to kill many other micro-organisms including different bacteria, viruses and eukaryotic cells. It can be purified from microbial fermentation and modified chemically or enzymatically. Antibiotics are secondary metabolites and these secondary metabolites have also been termed "idiolites" (Walker, 1974). The best-known genus of actinomycetes; Streptomycetes (Order Actinomycetales, Family Streptomycetaceae) are gram-positive, filamentous bacteria that are ubiquitous in soil and produce the majority (>70%) of known antibiotics (Tanaka and Omura, 1990). There is a large class of β-lactam antibiotics produced by bacteria, fungi and streptomyces. They all possess unusual chemical linkage β-lactam core (Bayer *et al.* 1990). Sometime it shows the "selective toxicity" which means that compound inhibits or kills the microorganism without having a similar effect on the host organism (e.g., humans) (Robbers *et al.* 1996). Streptomycin belongs to a group of compounds, known as antibiotics, which are produced by microorganisms and which possess the property of inhibiting the growth and even of destroying other microorganisms. Antibiotics vary greatly in their chemical nature, mode of action upon different organisms, and effect upon the animal body.

The selective action of antibiotics upon bacteria and other microorganisms is known as the antibiotic spectrum. The majority of antibiotics and substances with diverse biological activity used in medicine are produced by actinomycetes, however, other microorganisms, such as M*yxobacteria, Pseudomonads, Nocardias, Enterobacteria, Halobacteria, hyperthermophiles* etc. are investigated for new biologically active metabolites by Behal (2003). As soil bacteria can metabolize a variety of carbon source and their activity and regulation substantially determine the nutritional status of the cell and therefore, influence antibiotic production (Bertram *et al.* 2004) It has been established that penicillin production is characteristic of the Penicillium notatum and Penicillium chrysogenum groups; the variation in potency of different strains is either quantitative or qualitative, according to the type of penicillin produced. The production of streptomycin, however, is characteristic of only a certain few strains of S. griseus (Waksman, Schatz, and Reynolds, 1946). This organism represents a distinctly heterogeneous group, especially in regard to the production of antibiotics. Numerous attempts to isolate streptomycin-producing strains of S. griseus from natural substrates have so far yielded, in addition to the two original strains obtained in laboratory in 1943, namely, D-1 and 18-16 (Schatz, Bugie, and Waksman, 1944).

II. MATERIAL AND METHODS

Collection of soil samples : The soil sample was collected from Shalini park, Jayanagar 4th block Bangalore from a depth of 1-15 inches from the top and sieved through a 2 mm sieve constituted the soil sample. The sample was dispensed into bag and was brought to the laboratory.

Isolation of microorganisms : The saline soil solution was prepared by dissolving the 100mg of soil in 10ml autoclaved saline water. Now take the 1 ml of saline soil sample from that and pour it into the petripalate. After add the sterile media into petriplates. Wait for the solidification of the medium in petriplate. Allow it to incubate for 24-48 hour in incubator for 37ºC.

Identification of the bacterial cultures: Identification of bacterial cultures was performed by gram staining and by performing the biochemical test.

Production of Streptomycin: Production of Streptomycin was done by inoculating the bacterial cultures of S. griseus on to the Starch Casine Broth for 24-48 hours.

Antimicrobial Actvity Testing of the Streptomycin: Take 1 ml. of the streptomycin culture from different broths in the ependrop tubes. Centrifuge the ependrop tubes at 6000 rpm for 10 minutes. Take out the supernatant. Pour the nutrient agar medium into the petriplates. Allow it to cool at room temperature. Spread the *E. coli* and pseudomonas bacterial culture on to the surface of the medium. Make the wells in the medium of plate and fill the wells with the supernatant. Incubate the plates for 48-72 hours.

Estimation of Streptomycin: Estimation of Streptomycin was done with the help of HPLC.

Preparation of Mobile Phase: Mobile phase is 75% HPLC grade Acetonitrite: 25%HPLC grade water. Mobile phase prepared by using single cylinder measurement system.

Preparation of Sample: 1ml. of streptomycin sample is taken and diluted with 10 ml mobile phase.

Preparation of Standard: 10 mg of standard powder is taken and diluted with 25 ml of mobile phase.

Column Used: C18 is the column used.

Flow Rate: 1 ml/min.

Calculation: Sample Area÷Standard Area×Standard Amount÷Dilution×dilution÷Sample Amount×Mean of Sample of Streptomycin.

Optimization of medium for the production of Streptomycin: Optimization medium was done on the basis of pH, incubation time, carbon source, nitrogen source.

III. RESULTS

Isolation and Identification

Starch Casine Agar (SCA) media was employed for the isolation of bacterial colonies from the soil sample. The Bacterial strains were isolated from soil sample collected from Shalini Park Jayanagar 4th Block, Bangalore. Serial dilution of the soil sample was done with saline double distilled water. Grayish whitish colonies were grown on to the culture plates selected. Further morphological identification was done by gram staining. Gram positive purple colour filamentous bacteria were seen under the microscope. Biochemical identification of the isolate was performed (Shown in Fig.). The isolate was identified by morphological, biochemical and physiological examination according to Bergey's Manual of determinative bacteriology (1994). The organism was described to genus *Streptomyces* and species *griseus* (Table).

Fig. Showing the morphology of bacteria.

Fig. showing the Methyl Red Testing.

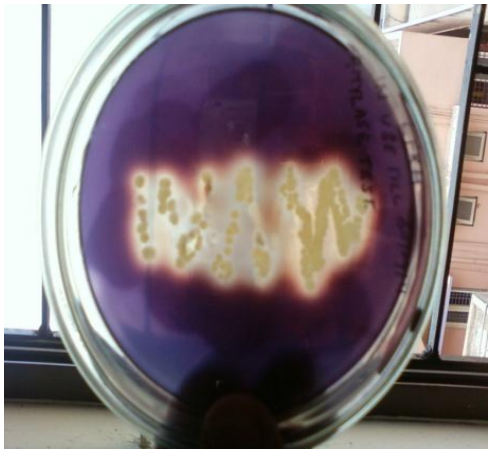

Fig. showing Amylase production.

Fig. Showing Glucose Fermentation.

1.	Amylase Test	+ve
2.	Fermentation of Carbohydrate	+ve
3.	Hydrogen Sulphide Production	-ve
4.	Casine Hydrolysis	+ve
5.	Urease Test	-ve
6.	Cellulase Production	-ve
7.	Citrate Utilization	-ve
8.	Catalase test	+ve
9.	Oxidase Test	-ve
10.	Gelatin Hydrolysis	-ve
11.	Indole Production	-ve
12.	Methyl Red(MR)	+ve
13.	Voges-Prokauer(VP)	-ve

Table: Biochemical testing of the isolated bacterial culture.

Antimicrobial activity of Streptomycin: A clear zone was formed around the well of the medium.

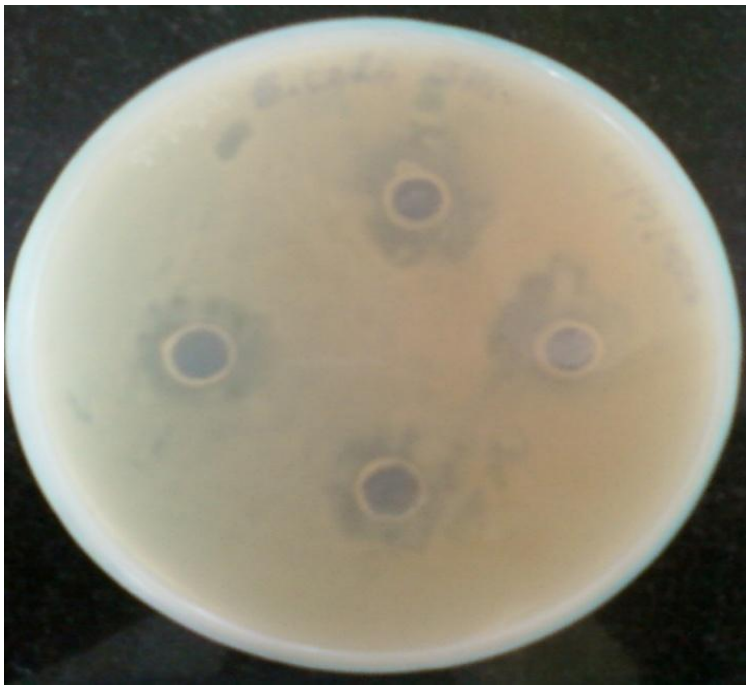

Fig. Showing the antimicrobial activity of streptomycin.

Estimation of Streptomycin by HPLC:

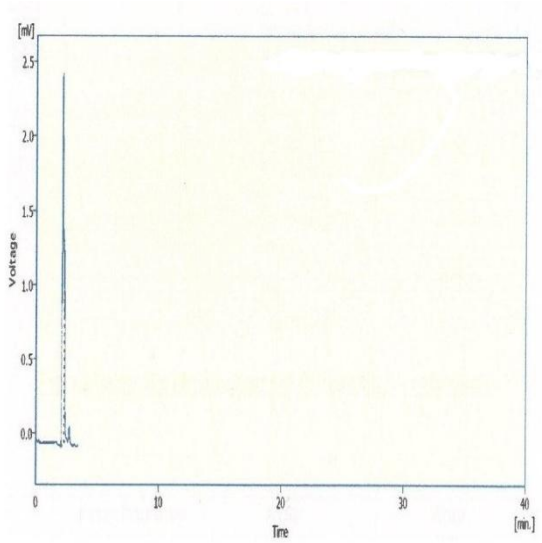

Fig. showing the chromatogram of Standard of Streptomycin

	Retention time (Min.)	Area (mV.s.)	Area (%)
1	2.453	24.720	100.0
	Total	24.720	100.0

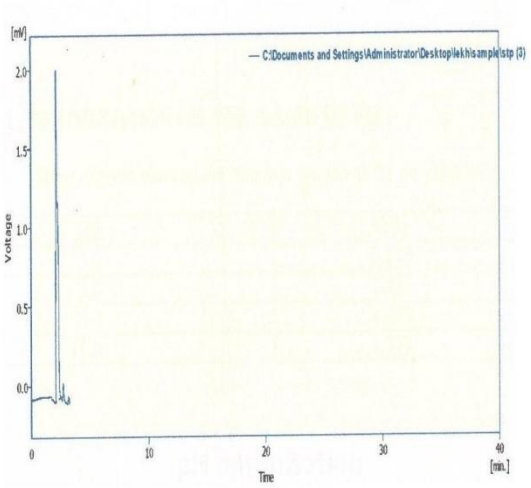

Fig. showing the chromatogram of Sample Streptomycin

	Retention time (Min.)	Area (mV.s.)	Area (%)
1	2.140	21.498	100.0
	Total	21.498	100.0

Calculation: 21.498÷24.720×1ml÷10ml×10ml÷1ml×10ml= 8.6966019 ml.

8.6966019 ml of Streptomycin sample was found in 10ml sample.

Optimization of medium: On the basis of pH:

Streptomycin showed the maximum growth at the pH value of 9.

pH	O.D.
5.0	0.875
7.0	1.217
9.0	1.613
11.0	1.373

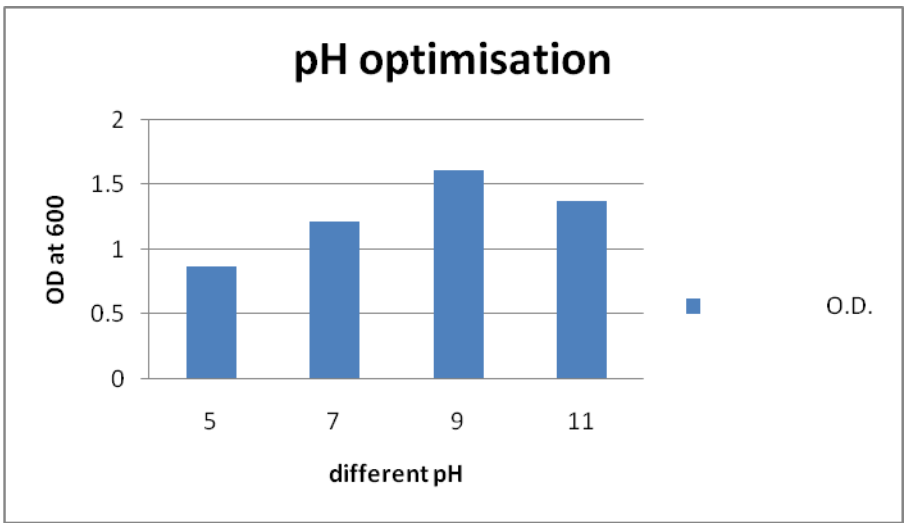

Fig. Showing the pH Optimization.

On the basis of Incubation time: Streptomycin showed the maximum growth when the incubation time of the culture is increases more than 72 hours.

INCUBATION TIME(HOURS)	O.D.
24	0.875
48	0.997
72	1.167
116	1.256

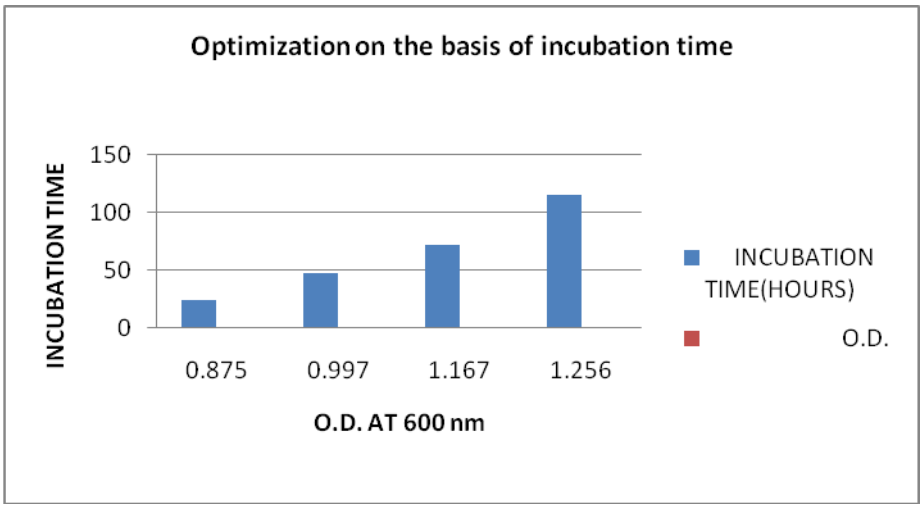

Fig. Showing the Optimization on the basis of incubation time.

On the basis of Nitrogen source:

Streptomycin gave the maximum yield or growth when nitrogen source glycin and peptones are used.

NITROGEN SOURCE	O.D.
PEPTONE	0.611
TRYPTONE	1.119
GLYCINE	1.509

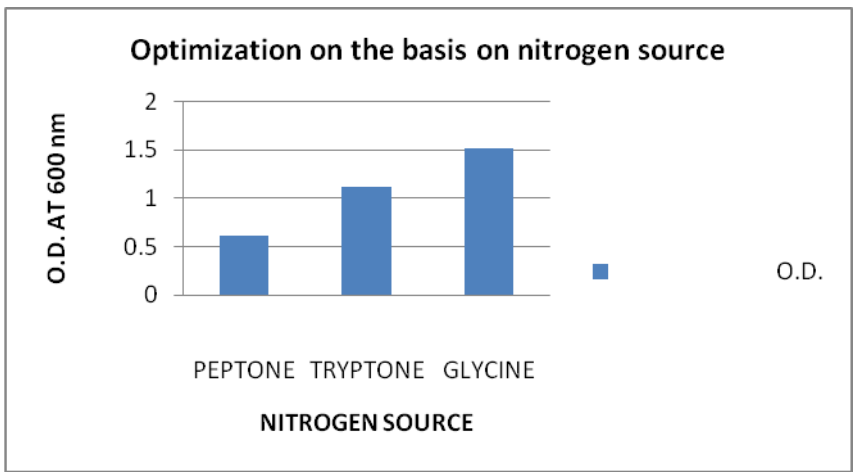

Fig. Showing Optimization on the basis of Nitrogen source.

On the basis of Carbon source:

Streptomycin gave the maximum growth when carbon source starch and rice bran are used.

CARBON SOURCE	O.D.
MALTOSE	0.541
STARCH	1.046
SUCROSE	0.883
WHEAT BRAN	0.971
RICE BRAN	1.119

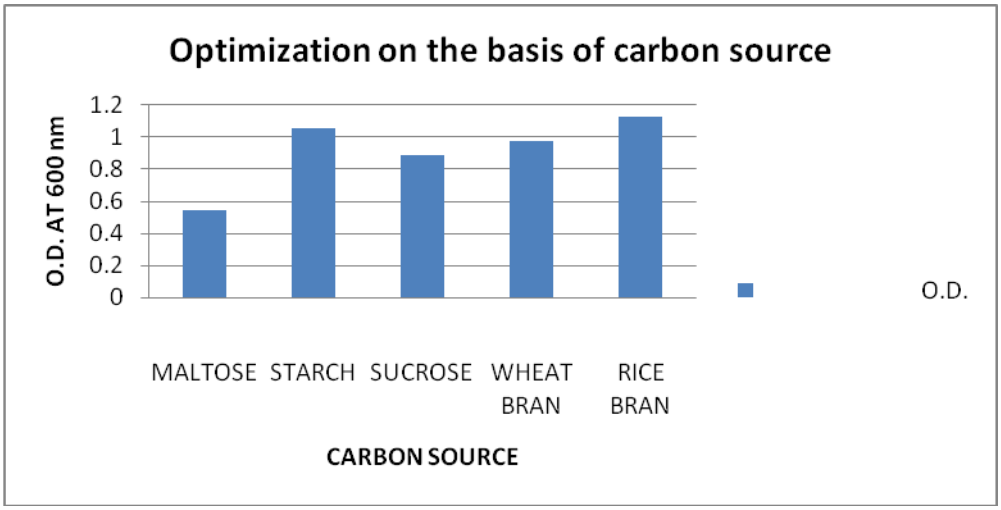

Fig. Showing Optimization on the basis of carbon source.

IV. CONCLUSION

Streptomycin was isolated from the soil sample. Various species of Streptomyces spices can be used for the isolation of the streptomycin. In this project *Streptomyces griseus* species was used for the production of streptomycin. The sample was collected from Jayanagar 4th block Shalini Park. Starch casine medium is used for the bacterial isolation which is the specific medium for *Streptomyces griseus*. Grayish or whitish colonies of the bacteria were observed. *Streptomyces griseus* bacteria was Filamentous, purple colour gram positive morphology of the bacteria is found. After performing biochemical test confirms the bacteria. In the next step is the production of the bacteria. Antimicrobial activity of the bacteria is checked against the pseudomonas and *E. coli*. Estimation of the streptomycin was done by the use of HPLC and the optimization of the medium was done for the Streptomycin production. Optimization was done on the basis of pH, incubation time, nitrogen source, and on the basis of carbon source. Different graphs of the optimization have been shown in this project work explaining the effect of growth in under different components. This work is important for getting good yield of Streptomycin from bacteria. In future more work is possible on it by increasing the yield of the Streptomycin by genetically modifing the strain of bacteria.

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REFERENCES

[1] Bayer, D., W. Zhou, K. Holzhauer and K. Schuegar. 1990. Investigation cephalosporin C production in an airlift loop towel loop reactor. Appl. Microbiol. Biotech. 30(1): 26-33.
[2] Behal, V. 2003. Alternative sources of biologically active substance. Folia. Microbiol (Paraha). 48(5): 563-571.
[3] Bertram, R., M. Schlicht, K. Mahr, H. Nothaft, M.H. Saier and F. Titgemeyer. 2002. Insilico and transcriptional analysis of carbohydrate uptake systems of streptomyces coelicolor A3 (2). J. Bacteriol. 186(5): 1362-1373.
[4] Rubbers, J.E., M.K. Speedie and V.E. Tyler. 1996. Pharmacognosy and Pharmacobiotechnology. Williams and Wilkina Co. 219p.
[5] Selman A. Waksman Streptomycin: background, isolation, properties, and utilization Nobel Lecture, December 12, 1952.

[6] Waksman, S. A., Schatz , A., AND Reynolds, D. M. 1946 Production of antibiotic substances by actinomycetes. N. Y. Acad. Sci., Conf. Antibiotics, Jan. 17-19.
[7] Schatz, A., Bugie, E., and Waksman, S. A. 1944 Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. Proc. Soc. Exptl. Biol. Med., 55, 66-69.
[8] Tanaka, Y. and S. Omura. 1990. Metabolism and products of actinomycetes: an introduction. Actinomycetologica 4: 13-14.

[9] Walker, J.B. 1974. Biosynthesis of the monoguanidinated inositol moiety of bluensomycin, a possible evolutionary precursor of Streptomycin. J. Biol. Chem. 249: 2397-2404.