STUDENT STUDY PROJECT (2017-2018)

Name of the Topic:

Isolation of antibiotic producing microorganisms from soil (Streptomycin).

Under the Guidance of

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Introduction

After Penicillin was discovered the search for additional antibiotics focused on the many fungi and bacteria that call the soil home

One particular family of microbe grabbed the attention of scientists the actinomycetes. This mouthful of name comes from the ancient Greek words for ancient Greek.

Even so, some scientists consider actinomycetes to be bacteria while others peg them as fungi. Still others think the actinomycetes are the prototype from which both bacteria and fungi are derived. Finally, some believe that the actinomycetes should be in a separate group between true bacteria and the filamentous fungi. In the final analysis, research investigations have placed the actinomycetes with the bacteria. Regardlesss, the soil-dwelling actinomy cetes give us a variety of antibiotics including streptomycin, aureomycin, terramycin, and chloromycetin. Actinomycetes are unicellular organisms that mass together to form filaments called hyphae. Colonies of actinomycetes can then form a mass of in intertwined hyphae called a mycelium.

In the activities that follow, you will attempt to isolate the hypae of actinomycetes that successfully grow on agar. You will also attempt to determine if any of the actinomycetes species have antibiotic properties. Finally, for those actinomycetes that appear to have antibiotic properties, there is a procedure for isolating the antibiotic compound. This procedure is a kind of fermentation, and it mimics the processes used by pharmaceutical companies to isolate antibiotics from fungi.

ISOLATION OF ACTINOMYCETES FROM SOIL

COLLECTION OF SOIL SAMPLES :

Soil samples were collected from three Indian states viz. Maharashtra, Karnataka and Kerala. The Samples were collected in sterile containers and maintained at 4^0C until analysis.

PROCEDURE :

1. Mass 1.0g of soil for each sample to be tested
2. Transfer to 9 cm3 of sterile water. This is a 1/10 dilution. SHAKE VIGOROSULY 50 times.
3. Perform a series of dilutions – 1/10, 1/100, 1/1000, 1/10,000 1/100,000, 1/1,000,000 (see notes on performing a soil dilution below)
4. Add 1.0 Cm3 samples of each of the dilutions, 1/100,000 and 1/1,000,000, to each of two petri dishes that have been sterilized previously.
5. To each of the dishes, add 10-15 cm3 of soil extract agar at approximately 45^0C. Immediately upon addition of the agar, the dishes are rotated by hand in a broad swiriling motion so that the inoculum is uniformly dispersed in the medium.
6. Allow the agar to solidify and then incubate the plates at 28^0C for 7 days.
7. After 7 days of incubation, when there is growth of organisms on the two sets of plates examine the petri dishes carefully. Hold them up to the light and look for clear zones or HALOS around actinomycetes colonies. The zone of inhibition may be small or the actionomycete colony may be completely surrounded by an area free of growth by other organisms.

SCREENING OF ANTIBIOTIC PRODUCING MICRO ORGANISAM

1. After 5 days, remove the plates and prepare to test the antibiotic production and effectiveness by adding streaks of various bacteria. To do this, you need to have solutions of various bacteria prepared form stock cultures.
2. Water Solutions of the various bacteria are made by transferring a sterile loop of the bacteria taken from a stock culture to a sterile test tube containing 5 cm^{3} of sterile distilled water. From this water solution, a loop of the bacteria is transferred to the nutrient agar plate containing the center streak of the antibiotic-producing.
3. For purposes of relating antibiotic effectiveness against particular bacterium, a collection of different bacterial types (Gram positive, Gram Negative) are suggested. They include Sarcina lutea(+), Serratia marcescens (-)as well as the yeast, Saccharomyces cerevisiae.
4. Incubate the plates at 28^{0} for 2 days.
5. Examine the plates for evidence of antibiotic activity against the various bacterial streaks. Is there any correlation between those bacteria that are affected by the antibiotic and their designation, Gram Possitive, Gram Negetive? Refer to literature that explains Gram staining results relative to the type of bacterial cell wall composition. How is this related to the activity of Streptomycin

STREPTOMYCIN

Introduction

Streptomycin is discovered first by Waksman and his team in 1944. They isolated the antibiotic from Streptomyces griseus.

The Nobel Prize in Physiology or Medicine 1952

In 1951 Dr. Waksman and one of his assistants had isolated from the soil a strain of actinomycete which they called Actinomyces griseus. This name was changed to Streptomyces griseus in 1943 and under this name it has now become world renowned. It is from strain of this species that streptomycin is produced. Dr. Waksman had shown that of the microbes, Streptomyces was best able to survive when the living conditions in thesoil became unsatisfactory, and this was an additional reason for commencing with the Streptomyces.

In 1940 Dr. Waksman and his collaborator had succeeded in isolating the first antibiotic, which was called <<actinomycin>> and it was very toxic. In 1942 another antibiotic was detected and studied, called <<streptothricin>>. This had a high degree of activity against many bacteria and also against the tubercle bacillus. Further studies revealed that streptothricin was too toxic. During the streptothricin studies Dr. Waksman and his collaborators developed a series of test-methods, which turned out to be very useful in the isolation of streptomycin in 1943.

The activity of streptomycin is principally bacteriostatic, i.e. it checks the bacterial growth and is in some degree also bacteriolytic, i.e. it destroys the tubercle bacillus. The mechanism of this important antibacterial effect is not yet known.

Chemistry of Streptomycin

Streptomycin is one of the aminoglycoside antibiotics. The aminoglycosides are the oligosaccharide antibiotics and consist an aminocyclohexanol moiety which is linked glycosidically to other amino sugars.

Medical use of Streptomycin

Streptomycin is particularly active against Gram-negative bacteria and Mycobacterium tuberculosis. It is used in therapeutic treatment of infections caused by organisms which are resistant to penicillin. Streptomycin also acts as systemic antibiotic in the treatment of some plant diseases caused by bacteria. The prolonged treatment with streptomycin high dosages results in neurotoxic reactions and partial hearing loss in man.

Activity

The general process of protein synthesis involves the binding of Ribosome to m-RNA

Streptomycin recognizes 30s subunit of bacterial Ribosome thus it inhibits the binding of Ribosome to the m-RNA & No more the protein synthesis occurs. The recognition of the Streptomycin to Ribosome is specific in killing Gram-Negative bacteria mostly and Norcardia and tuberculosis bacillus.

Industrial Production of Streptomycin

Industrially, the antibiotic Streptomycin is known to be produced only from different strains of Streptomyces griseus.

Media Composition

Majority of the media employed in commercial production of Streptomycin are more or less similar in their composition. They commonly consist soyabean meal, Glucose and Sodium Chloride but at different concentrations

Glucose	10g
Soyabean meal	10g
Peptone	5g
Meat extract	5g
Sodium chloride	5g

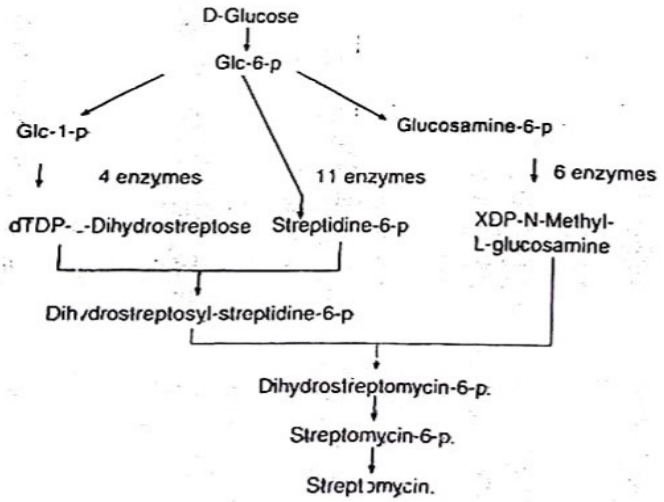
Inoculum

For the production of streptomycin, spores of Streptomyces griseus are normally used as inoculum.

The stock cultures of Streptomyces spores are usually maintained as soil stocks or are lyophilized with a carrier substance such as sterile skim milk. Initially, spores are inoculated into sporulation medium in which spores germinate to build up mycelial inoculum.

The fermentative production of Streptomycin lasts for 6 to 7 days and yields a maximum of 1200 micrograms per milliliter. High aeration and agitation of medium strongly influence the streptomycin yield. The temperature between 25°C to 30°C and pH between 7.6 to 8.0 are optimum for streptomycin production. But at 28°C temperature and at pH 7.6, Streptomycin production occurs at highest rate. The streptomycin produced is not destroyed by the microorganisms that occur as contaminants during fermentation process.

Chemistry of Streptomycin formation : The Convertion of D-glucose into streptomycin involves several enzymes.



Recovery

Usually, harvest is carriedout before the start of the senescence phase of the fermentation. After the completion of fermentation, mycelium is separated from the broth by filtration and Streptomycin is finally recovered. The mode of antibiotic recovery differs among the industries. In one of the procedures, Streptomycin is adsorbed from the broth onto activated carbon. From this activated carbon, Streptomycin is eluted with dilute acid. The Streptomycin is then precipitated by solvents, fitered and dried before further purification.

In an another procedure, the fermentation broth is acidified filtered and neutralized. Then it is passed through a column containing a cation exchange resin to adsorb the Streptomycin from the broth. Then the column is washed with water and the adsorbed streptomycin is extracted with HCl. Then it is dissolved in methanol and filtered. To this fitrate, acetone is added to precipitate the antibiotic. This precipitate is once again washed with acetone and dried in vaccum. It is then dissolved in methanol for preparation as pure Streptomycin calcium chlorided complex.

Secondary products

During the Streptomycin fermentation by Streptomyces griseus, small amount of vitamin B_{12} is also produced in addition to Streptomycin. The leval of vitamin B_{12} production can be markedly increased by adding a soluble cobalt salt to the medium as precursor, without affecting the yield of Streptomycin. But the concetration of cobalt salt should be at non-toxic level to Streptomycin production. The vitamin B_{12} that is produced as an additional product during Streptomycin fermentation process can be recovered from broth and used as supplement to animal feed.