



## EXTRACTION OF FLAVOURING COMPOUND FROM ACTINOMYCETES

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### ABSTRACT

The main objective of the present study was isolation, purification, and characterization of actinomycetes from soil samples, having a secondary metabolite production. Soils samples were taken from agricultural land. These samples were serially diluted and plated, on isolation of Actinomycetes agar media. Potential colonies were screened, purified, and stored in. Isolates were morphologically and biochemically characterized. These isolates were subjected to extraction for production of the Geosmin a chemical compound. Culture dependable isolation techniques were followed for the isolation of the active actinomycetes isolates and the secondary metabolite properties of the actinomycetes were detected by modified Tryptic Soy Broth, and the distillation takes place. Then the crude extract of geosmin is separated using distillation method.

**Key Words:** - Geosmin, Isolation of Actinomycetes from soil, Chromatographic techniques, Extraction process.

### INTRODUCTION

Geosmin is a natural bicyclic terpene with an earthy odour. The name geosmin is derived from the Ancient Greek words - Geo, meaning "earth", and osme, meaning "smell". The word geosmin was coined in 1965 by the American biochemist Nancy N. Gerber (1929–1985) and the French-American biologist Hubert A. Lechevalier (1926– 2015). There is another name for geosmin which is “petrichor”. The word *petrichor* comes from the Greek, Petros, meaning ‘stone’ + *ichor*, the fluid flowing in the veins of the gods in Greek mythology. It is a trans-1,10-dimethyl-



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*trans*-9-decalol, in chemical terms the geosmin is also called a bicyclic alcohol with the formula of  $C_{12}H_{22}O$  is a dicyclohexyl ether, it is a derivative of decalin.

The Geosmin is expressed when the rain hits the earth, it is responsible for the muddy smell while rain comes. When a raindrop lands on earth at a porous surface, the air from the pores forms a tiny bubble-like structure, which appears on the surface and release aerosols. An aerosol is a suspension of fine solid particles or liquid droplets in air. Such aerosols carry the scent through air, as well as the bacteria and viruses from the soil. The raindrops that move at a slower rate can able to produce more aerosols, this gives us an explanation for why the petrichor is more common after light rains.

The Actinomycetes is the bacterium responsible for producing spores in soil, which releases the geosmin a metabolite which is responsible for the pleasant earthy odour. There are lot of animals are sensitive to this smell, but human nose is highly sensitive to the geosmin compound and we can able to detect it from a low concentration as low as **400 parts per trillion**. There is something primitive and primal about the smell, some scientists believe that human appreciate the smell of rain because of our ancestors, they may have relied on rainy weather for survival.

*Actinomycetes* are prokaryotic organisms they are classified as bacteria, but they are unique and to be discussed as an individual group. They are the important component of the bacterial community, especially under high pH condition. Morphologically, *Actinomycetes* resemble fungi because of their elongated cells that branch into filaments or hyphae. These hyphae can be distinguished from fungal hyphae on the basis of size with actinomycete hyphae much smaller than fungal hyphae. Characteristics and One distinguishing feature of this group of bacteria is that they are able to utilize a great variety of substrates found in soil, especially some of the less degradable insect and plant polymers such as chitin, cellulose and hemicellulose.

## Materials and Method

### Sample Collection:

The soil samples are collected from various land surfaces like mountain soil, agricultural lands. The collected soil samples were sundried for 2 days, to kill most of Gram-negative bacteria. The soil is further heated in a hotplate at  $60^{\circ}C$  to further destroy other vegetative microorganisms. 1g of soil sample is taken to prepare stock solution.

### Isolation of Actinomycetes

Isolation of Actinomycetes was done by serial dilution and spread plate technique. For the isolation, Starch Casein Agar media was taken. The spreaded plates were incubated at  $28^{\circ}C$  for 7 days. After 7 days, the inoculated plates are examined and *Actinomycetes* are isolated based on morphology of the colonies formed on the surface. A whitish pin-point colony, characteristic of actinomycetes, with a clear zone of inhibition around them were observed. The whitish pinpoint colonies with inhibitory or clear zone of inhibition were selected and purified.

### Isolation of Actinomycetes (Pure Culture):

After 7 days, the inoculated plates are examined and *Actinomycetes* are isolated based on

morphology of the colonies formed on the surface. And then, the isolation of *Streptomyces* spp from *Actinomycetes* takes place. To isolate *Streptomyces* spp, the colonies are selected and inoculated in a Petri plate using an inoculation loop in a sterile condition. This isolation is done using Quadrant streaking Method. And the plates are incubated at 28°C for 72 hours.

#### **Mass Culturing of *Streptomyces* spp: -**

The isolated *Streptomyces* spp. transferred to Erlenmeyer flask containing Tryptic Soy Broth. And then, the flask is closed with cotton gauge and wrapped with aluminium foil. The culture is kept in a shaking incubator or Fermenter at 28°C for 72 hours.

#### **Characterisation of Isolated spp**

To confirm the microorganism isolated is *Actinomycetes*, the Gram staining and biochemical tests Indole Test, Methyl Red Test, Voges-Proskauer Test, Triple Sugar Iron Test, Citrate Utilization Test and Starch Hydrolysis were done. Test are successfully completed and the results were tabulated.

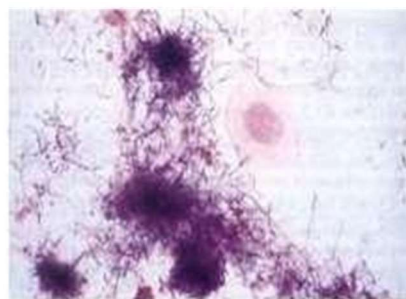
#### **Goemin Extraction:**

The whole broth was distilled at atmospheric pressure until 20% of their volume had been collected. The crude Geosmin was collected and stored, and then for further purification and extraction. The collected distillates undergo Gas - Chromatography technique. The average level of geosmin production in fermenter's was 6 mg/litre. Geosmin can be obtained in pure form by preparative gaschromatographic technique, pure geosmin is obtained with approximately 1 mg to 6 mg per litre.

## **RESULTS**

#### **Isolation of *Actinomycetes* -**

The powdery form colonies were formed after 7 days. A whitish pin-point colony, characteristic of actinomycetes, with a clear zone of inhibition around them were observed. From the morphological characteristic, it is observed as *Actinomycetes*. From the colonies appeared on the petri plates, selected particular colonies were taken and streaked on SCA to isolate the pure culture, using Quadrant streaking Method and subjected to Gram Staining, Biochemical characterization respectively.



**Figure 1: *Actinomyces spp.* (Spread plate Method)**

**Figure 2: Gram Staining (Positive)**

**Table 1: Biochemical Analysis of *Actinomyces spp.***

Test	Result
Gram Staining	Positive
Indole Test	Positive
Methyl Red Test	Positive
Voges-Proskauer Test	Positive
Triple Sugar Iron Test	Positive
Citrate Utilization Test	Negative
Starch Hydrolysis Test	Positive

**Mass Culturing of *Streptomyces spp.* –**



**Figure 3 : Tryptic Soy Broth containing *Streptomyces spp.***

**Goemin Extraction –**

After 3 days, the flask is taken out, and the liquid culture is taken for distillation process. It is

distilled until and collected 20% of distillates from it. The culture is heated because the *Streptomyces spp.* Produces spores which possess the secondary metabolite compound Geosmin. The distillates were collected and it is known as the crude extract of Geosmin. Then the distillates were purified and extracted using GC – Gas chromatography. The pure form of geosmin is extracted and it can be used in perfume industries for fragrances in perfumes and Airfreshners.



**Figure 11: Crude Geosmin Extract**

## DISCUSSION

There are number of studies discussed in this review, the use of flavouring compounds in perfume industries. The fragrances extracted from microorganisms which results in the reduction of indoor air pollutants which causes life threatening diseases. The perfumes and other fragrances producing industries estimated around \$3 billion usd. The production cost of these products is high due to the usage of chemicals and also the natural flavouring products. The usage of fragrance can come from a number of sources like Scented cleaning products, Fragrance-emitting devices and sprays, Workers, patients, and visitors who are wearing perfume, cologne, or aftershave, Scented cosmetics, skin lotions, or hair products, Clothes that have been laundered with scented detergents, fabric softeners, or dryer sheets. In these ways the perfumes and other related products are used. By involving microorganisms in production of fragrances, it leads to a massive production also it is cost effective and harmless.

The Goemin compound is extracted in a crude form and for future studies it is purified and extracted using Gas – Chromatography techniques. And the pure form of Geosmin is isolated and it can be used as a “Rainy Earth Fragrance” in the perfume industries to produce Airfreshners without any toxic chemicals. A study shows that, the smell of rain reduces stress levels, anxiety and feel fresh. The human nose is more sensitive to geosmin compared to other animals.

## CONCLUSION

Nowadays everyone using perfumes, airfreshners which is basically composed of chemicals. These chemicals are used to make different fragrances and flavours, but these chemicals are highly toxic and causes life threat to the long-term users. Air fresheners emit over 100 different chemicals, including volatile organic compounds (terpenes such as limonene, alpha- pinene, and

beta-pinene, terpenoids such as linalool and alpha-terpineol; ethanol, formaldehyde, benzene, toluene, and xylene) and semi-volatile organic compounds and semi-volatile organic compounds. The chemicals in it are undisclosed substances some of which are linked to reproductive and developmental toxicity. The chemicals like formaldehyde, toluene, phthalates, styrene etc. Which causes cancer, reproductive problems, hormone disruption etc. To avoid these side effects caused by various chemicals involved in the production of fragrances and flavours, With the help of our friendly microbes we can able to produce different kinds of fragrances and flavours, which have no side effects and cost-effective method too. Here in the study actinomycetes were isolated from agriculture soil sample. And the secondary metabolite of actinomycetes goemin (a flavouring compound) were extracted crude extract of goemin is distilled out.

The natural flavouring perfumes and air fresheners are also not 100% natural but the production cost is more and the natural fragrances is composed of essences which is extracted from lot of flowers and fruits. The fruits and flowers are grown in particular season's based on the climatic conditions etc. So, the fragrances extracted from these kinds of fruits and flowers are very less and the demand for this product are high. By involving microorganisms into the production flavours and fragrances we can able to reduce the cost of the production. So, as a flavoring agent Goemin can be recommended.

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