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Studies on Isolation and Identification of Streptomycete's Carotenoides

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Abstract: Carotenoids are natural pigments synthesized by plants and microorganisms. The present study to isolates Streptomycetes spp from the carotenoids samples by spectroscopic and TLC methods. These pigments are not essential for other carotene-containing microorganisms, as is the case for fungi, but they are indeed very important due to their ability to act as antioxidant agents.

Key words: Carotenoids · *Streptomycetes* spp · Spectroscopic and TLC methods

INTRODUCTION

Triterpenoids and their precursor isoprenoid hydrocarbons are among the oldest known and most ubiquitous chemicals on the earth, having been found in fossil remains from a variety of geological formations and sediments [1-2]. The reconstruction of biosynthetic pathways from such records indicates that the ability to synthesize isoprenoids such as pristane and phytane and later, triterpenoids such as squalene and hopanes was present in non-photosynthetic bacteria before the evolution of chlorophyll biosynthesis. The occurrence of triterpenoids in bacteria and especially in non-photosynthetic bacteria, is thus of interest from both evolutionary and functional aspects.

Carotenoids are natural pigments synthesized by plants and microorganisms, but not by animals. Carotenoids dare classified as follows: 1) Carotenoid hydrocarbons are known as carotenes and contain specific and groups. Lycopenes have two acyclic end groups. β-Carotene has two cyclohexene type end groups. 2) Oxygenated carotenoids dare know as xanthophylls. Examples of these compounds are a zeaxanthin and lutein (Hydroxy, b) spirilloxanthin (methoxy, c) echinenone (oxo) and d) antheraxanthin (epoxy) [3].

The concentration of each carotenoid in a fruit or vegetable suggests which enzyme or enzymes may be rate-limiting in the biosynthetic cascade. For example a very high concentration of lycopne in red tomatoes suggests a lack of sufficient enzyme activity to convert lycopene to g-carotene (i.e. insufficient cyclase activity) [4]. Of the over 600 carotenoids found in nature, about 40 are present in a typical human diet. Of these carotenoids, only 14 and some of their metabolites have been identified in blood and tissues [5]. Many epidemiologic studies have associated high carotenoid in take with a decrease in the incidence of chronic disease. However, the biological mechanisms for such protection are currently unclear.

The present study to isolates streptomycetes spp. from the carotenoids samples separated from the streptomycetes spp. by (i) quantification of various carotenoids spectroscopic analysis of carotenoids and (ii) qualitative estimation of carotenoids TLC analysis of carotenoids. Various pigments were obtained from the carotenoids. These pigments act as protective agents against oxidative damage and act as antioxidant agents.

MATERIALS AND METHODS

Estimation of Microbial Population: In the present experimental work, the microbial population (bacterial, fungal and Streptomyces) were enumerated. The media were employed to isolate *Streptomyces spp* from the samples. Total microbial populations were estimates by pour plate method. The total number of Streptomycete colonies in each petri-dish was counted and the mean number was divided by the dry-weight of the sample (sediment) and then multiplied by the dilution factor to obtain the total number of microbes per gram of sample.

Isolation and Maintenance of *Streptomyces* spp Culture: The streptomycete colonies appeared in the media were isolated at random and sub cultured on Glycerol Asparagine Agar. The stock cultures were periodically

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sub cultured and stored at 4°C for further studies. The pigmentation of aerial and substrate mycelia was noted. Isolation and mass culture of the selected strains were carried out in Glycerol Asparagine broth. The pure culture of Treptomyces spp was inoculated in Glycerol Asparagine broth medium and incubated for 14 days. The mass culture suspension of *Streptomyces* spp was filtered, homogenized and added to the feed.

Systemic Study of *Streptomyces* spp: The International Streptomyces Project (ISP) was initiated in 1964 to overcome the problem in Streptomyces taxonomy. The important characters which are taken into consideration for the classification and identification are colour of spores, spore morphology, spore surface ornamentation [6].

Extraction and Separation of Carotenoids: To the pre-weighed samples of about 5grams added with cold Acetone solvent and homogenized well. Then the solvent petroleum ether was taken in a separating flask (500 ml of about 50 ml and then acetone extract samples were transferred. Slowly add distilled water along the sides of the separating flask (300 ml). You will observe the two phases getting separated. Collect the upper phase that contains petroleum ether and carotenoids. Then it was stored in – 20°C.

Spectroscopic Analysis of Carotenoids: The samples extracted with the petroleum ether solvent were scanned between 350 and 500nm using UV – Vis Scan Spectrophotometer and it shows individual peaks for respective carotenoids with their optical denisites.

Estimation of Total Carotenoid: The extracted carotenoid from each experimental sample was diluted to approximate volume as to be obtaining the optical density value for that the solvent used for the carotenoid extraction was used. After proper dilution, the optical density was measured at 400-500nm. Total carotenoid in the sample was then estimated by using the formula given below.

$$\text{Total carotenoid content } (\mu g/g) = \frac{\text{A x volume (ml) x 10}}{\text{A1\% 1 cm x sample weight (g)}}$$

Qualitative Estimation of Carotenoid

TLC Analysis of Carotenoids: Qualitative analysis of carotenoid in the experimental sample was carried out by using Thin Layer Chromatography (TLC). Then, applying slurry made by silica Gel G for TLC grade and applied over the glass plate, TLC plates were made. This was dried at 60°C for an hour of period. The dried plates were pre activation base line was drawn on the TLC plate 3.0cm away from the base line on the portion of the TLC plate.

After that, 3µ 1 condensed carotenoid samples were spotted on the baseline of the TLC plates at 1.0 cm interval and then allowed to dry at room temperature. Often the sample applied TLC plates was placed in a pre-saturated TLC chamber contains mobile phase. The mobile was placed used was 5% methanol/Toluene in the ratio of 95.5 (9v/v). Then the chromatogram was developed by providing the dark environment up to a distance of 15 cm mark. Then the plate was taken out dried for few min. Using U.V. light torch, the developed spots were seen and taken out and marked. The distance travelled by each spot in baseline and relative R_f values were calculated. By comparing the standard R_f values for the chosen mobile phase, the carotenoids present in the samples were identified.

$$R_f = \frac{\text{Distance travelled by the Substance}}{\text{Distance travelled by the solvent}}$$

RESULTS AND DISCUSSION

The microbial population of isolated strain was showed in Table 1. Since selective medium (GAA) containing glycerol was used, the number of bacterial and fungal colonies found were minimum. Ten different Streptomycetes strains were isolated from the soil sample collected from station 1, whereas station 2 and 3 gave 1 and 2 strains respectively. Out of these, four different coloured strains were selected for study because they showed better colouration. Mycelial colour of selected strains (AQB-A_1, AQB-A_2, AQB-A_3, AQB-A_4) of *Streptomyces* spp, isolated from soil sample. The four different strains showed different aerial and substrate mycelial colourations like white and brown, creamy white and organge, grey and yellow and grey and yellow respectively. In that bacterial and fungal colonies were minimum in numbers because of these selective medium (Glycerol asparagine agar) has glycerol in the medium, which inhibits the growth of bacterial and fungal population [7].

This maximal occurrence of *Streptomyces* spp colonies inhibited the growth of bacteria because it has already been proved that marine *Streptomyces* spp synthesized antibiotics, anticancer agents, L-asparaginase enzyme as reported earlier [8-9]. Colour study is one of the important characteristics of Streptomyces.

Table 1: Microbial population isolated from soil of Akkulam Lake

S. No.	Medium used	Source	Stations	CFU x 104 g dry weight			Selected
1	GAA	Soil	ST1	10	-	-	2
2			ST2	1	-	-	1
3			ST 3	2	-	-	1

GAA – Glycerol Asparagine Agar

Table 2: Mycelial colour characteristics of selected strains (AQB-A?, AQB-A2, AQB-A3, AQB-A4) of *Streptomyces* spp in selective media, isolated from soil samples of Akkulam Lake

		Glycerol Asparagine Agar medium	
S. No.	Strains	Aerial mycelium	Substrate mycelium
1	AQB-A_1	White	Brown
2	AQB-A_2	Creamy white	Orange
3	AQB-A_3	Grey	Yellow
4	AQB-A_4	Grey	Yellow

Table 3: U.V. Vis spectrophotometer analysis carotenoids of selected strains of Streptomyces

S. No.	Samples	Maximal peak Wavelength (nm)	Absorbance	Carotenoids	Total volume µg/100 g
1	AQB-A_1	425	0.7958	ζ– Carotene	3.07
2	AQB-A_2	470	0.5780	Lycopene	2.22
3	AQB-A_3	450	0.4512	B-Carotene	1.74
4	AQB-A_4	470	0.5039	Lycopene	1.94

Table 4: Thin layer chromatrography analysis of carotenoids of selected strains of Streptomyces

S. No.	Samples	Rf values	Carotenoids
1	AQB-A_1	0.90	ζ– Carotene
2	AQB-A_2	0.74	Lycopene
3	AQB-A_3	0.93	B-Carotene
4	AQB-A_4	0.74	Lycopene

The mycelial colour characteristics of selected strains of *Streptomyces* spp was seen in Glycerol asparagine media (Table 2). The Aerial mycelial colour expressed was white, creamy white and grey. The substrate mycelial colour shows brown, orange and yellow. These showed that different colouration may be due to medium provided with different macro and micro nutrients, which leads to more secondary metabolite production and expression.

The U.V. spectral analysed in the range of 250 – 550 nm results were showed in Table 3. These clearly indicated that each strain shows almost similar pattern of spectral data. The predominant carotenoid was found to Lycopene. Some specific carotene – Carotene was also found. This carotenoid was found in some *Streptomyces* species as per earlier reports. The biosynthesis of these carotenoids is restricted to green photosynthetic bacteria and a few actinomycetes. Among them *Streptomyces griseus* has been used to study the genes involved in this pathway. Five genes out of seven of two adjacent operons in one cluster could be identified to be sufficient for the synthesis of carotenoids [10]. The total carotenoids were found to be maximum in Streptomyces isolated strain AQB-A? and the least accumulation was found to be in Streptomyces isolated strain AQB-A_3.

The TLC results showed the presence of Lycopene, ζ – Carotene, β-Carotene, These all carotenoids picture cannot be shown because the visibility is only through U.V. light detector since the sample carotenoids concentration is less (Table 4). These results very well correlated with the results [11].

CONCLUSION

As far as the U.V. spectrum and TLC results concerned the biosynthesis of carotenoids in various strains of Streptomyces clearly indicate the presence of

Lycopene, β-Carotene, ζ– Carotene. These pigments act as protective agents against oxidative damage [12], are responsible for the color of many plants and animals [13] and are precursors of phyto hormones [14]. Some related compounds of β-Carotene, such as vitamin A, retinal and retinoic acid, have important roles in vision, nutrition and cellular growth and development.

Carotenoids are also important due to their potential antitumor properties [14] and because they are used as colorants in the food industry to pigment salmon, trout and poultry flesh, or to intensify the colour of egg yolk [15]. Carotenoids typically consist of a C40 hydrocarbon backbone, in the case of carotenes, often modified by different oxygen-containing functional groups, to yield cyclic or acyclic xanthophylls. The absorption properties of each carotenoid depend on the degree of conjugation and isomerization state of the backbone polyene chromophore. Compounds with at least seven conjugated double bonds can absorb visible light.

In the microbial world, carotenoids are present in both anoxygenic and oxygenic photosynthetic bacteria and algae and in many fungi [16, 17]. Carotenoids are essential for organisms with oxgenic photosynthesis (plants, algae, cyanobacteria) because of their protective role, which consists of both depleting the energy from chlorophyll and accepting it from other molecules, such as the reactive forms of oxygen. These pigments are not essential for other carotene-containing microorganisms, as is the case for fungi, but they are indeed very important due to their ability to act as antioxidant agents [18-19].

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