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Characterization of carotenoids from selected strains of *Streptomyces* sp.

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ABSTRACT

Characterization of carotenoids from selected strains of Streptomyces sp. were performed in Estuarine (Oneochromis mossambicus- Etroplus maculates) and Hymavathy Pond (fresh water) sediment samples. The UV spectrum, TLC and HPLC results showed the biosynthesis of carotenoids in various strains of Streptomyces sp., clearly indicate the presence of phytoene, phytofluene, α-carotene, β-carotene and β-isonenieratene. In the microbial world, carotenoids are present in both anoxygenic and oxygenic photosynthetic bacteria, algae and in many fungi. Carotenoids are essential for organisms with oxygenic 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.

Keywords: Gut of fishes, Carotenoids, Streptomyces sp.

INTRODUCTION

Carotenoids are natural pigments that are structurally very diverse yet similar in their general chemical structure and one widely distributed in nature where they fulfill essential biological functions. Carotenoids are pigmented compounds that are widely produced by both eukaryotes and prokaryotes [1]. They are synthesized by plants, algae and by some fungi and bacteria. They are involved in photosynthesis as accessory pigments, functioning as antioxidants light protection pigments and membrane stabilizers over 600 different carotenoids are known at present[1]. Carotenoids are lipophilic pigments with a yellow or red colour which originate from the terpenoid biosynthetic pathways that consist of a polyene hydrocarbon chain derived from eight isoprene units.

They are essential in photoautotrophic organisms, where they are located in the thylakoid membrane participating in the light harvesting process[2] as well as for protection against photooxidative damage [3].

Streptomyces sp. are chemoheterotrophic soil bacteria belonging to the order *Actinomycetales. Streptomyces* sp. are saprophytic filamentous Gram positive bacteria inhabiting particulate soil ecosystem and marine sediments throughout the world. Secondary metabolites from *Streptomyces* sp. contribute major products for pharmaceutical uses.

Streptomyces sp. are considered exceptionally well endowed for "chemical warfare" presumably allowing them to eliminate bacterial and fungal competitors in soil ecosystem. Although thousands of antibiotics have been isolated from *Streptomyces* sp. they represent only a small fraction of the repertoire of bioactive compounds[4,5]. Considering the above facts which lead to the studies on characterization of carotenoids from selected strains of *Streptomyces* species.

MATERIALS AND METHODS

Sample Collection: In the present investigation *Streptomyces* sp. population were isolated from gut of Estuarine – (*Oreochromis mossambicus - Etroplus maculates).* Hymavathy pond (fresh water) sediment samples.

Isolation : The gut of the fishes and the sediments were removed aseptically and transferred into the sterile flask and the serial dilution was carried out independently for each sample. One ml of the sample from appropriate dilution was pipette out into the sterile petridish. Fifteen ml of the Glycerol Asparagine agar medium were poured into the same Petridish and mixed thoroughly by rotating the Petridish both clockwise and anticlockwise directions and then incubated at room temperature (28±2°C) for 20 days.

Colour determination from different media: The media used for the colour determinations were Nutrient agar, Tryptophan medium, Actinomyces agar, Glucose yeast extract agar and *Streptomyces* medium. Microplates were prepared observed every day.

Carotenoid Extraction: To the pre-weighed samples of about 5 gs 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 added distilled water along the sides of the separating flask (300 ml). The two phases getting separated. Collected the upper phase that contains petroleum ether and carotenoids. Then it was stored at 20°C.

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

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 were used. After proper dilution, the optical density was measured at 400-500 nm. Total carotenoid in the sample was then estimated by using the formula given below:

$$\text{Total carotenoid content } (\mu g/g) = \frac{A \times \text{Volume (ml)} \times 10^4}{A^{1\%}\ 1cm \times \text{sample weight (g)}}$$

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.

After that, 3µl condensed carotenoid samples were spotted on the baseline of the TLC plates at 1.0cm interval and then allowed to dry at room temperature. Often the sample applied on TLC plates was placed in a presaturated TLC chamber contains mobile phase.(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 15cm mark. Then the plate was taken out dried for few min. Using UV 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.

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

HPLC analysis of Carotenoids: To the pre-weighed samples of about 5 g 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 added distilled water along the sides of the separating flask (300 ml) and observed the two phases getting separated. Collected the upper phase that contains petroleum ether and carotenoids. Concentrated the carotenoid solution at 35°C in rotary evaporator. Then it was re-dissolved in HPLC grade acetone solvent. As far as HPLC chromatographic condition C_{18} column is needed with mobile phase of mixture Acetonitrile: Methanol: Ethyl acetate (80:10:10) and at the flow rate of 1ml/ minute and it was UV detector.

RESULTS AND DISCUSSION

The present investigation was an attempt to understand the distribution pattern of *Steptomyces* sp. in the micro-environment of gut regions of fishes of two environmental biotopes viz (1) Estuarine (*Oreochromis mossambicus* (Tilapia), *Etroplus maculates* (Pearl spot)], (2) and Hymavathy pond sediment samples. The primary isolation of *Streptomyces* sp. was carried out under selective media like Glycerol asparagine[6]. In that bacterial and fungal colonies were minimum in numbers because of these selective medium (Glycerol asparagines agar), which inhibits the growth of bacterial and fungal population. This occurrence of *Streptomyces* sp. colonies inhibited the growth of bacteria because it has already been proved that marine *Streptomyces* sp. synthesized antibiotics, anticancer agents, L-asparaginase enzyme as reported earlier[6,7].

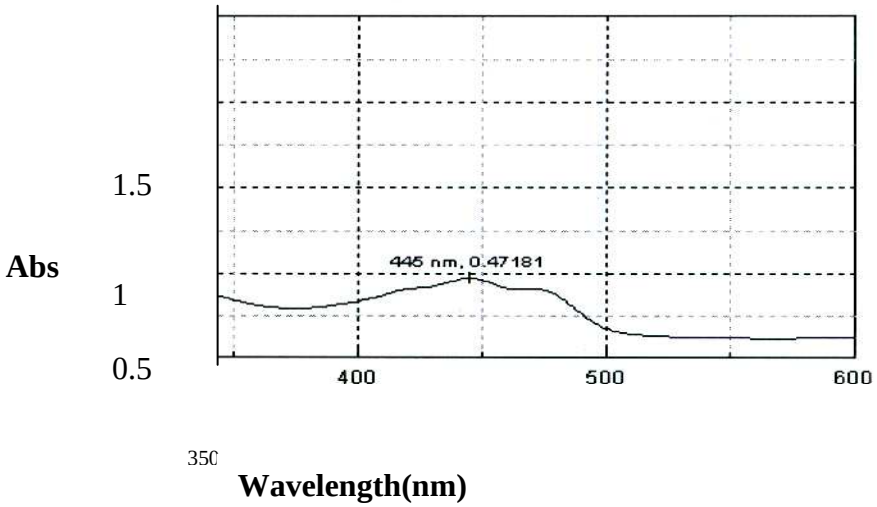
Mycelial colour characteristics in microplates of selected strains of *Streptomyces* sp. in different media like Nutrient agar, Actinomycetes agar, Glucose yeast agar, Tryptophan medium, Streptomyces agar medium shows the colouration pattern of aerial and substrate mycelia were

totally different in each medium with enrichment. This results has been observed for 14 days, the colouration pattern were entirely different in 7th day and 14th day.

The UV spectral analysed in the range of 400-600 nm results were showed in Fig 1 a,b,c. This clearly indicated that each strain shows almost similar pattern of spectral data. The predominant carotenoid was found to β-Carotene. Some specific carotene β-Isorenieratene was also found. This aromatic carotenoid was found in some *Streptomyces* sp. as per earlier reports. The biosynthesis of the aromatic carotene isorenieratene 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 isorenieratene[8]. The total carotenoids was found to be maximum in *Streptomyces* sp. isolated from gut of carp fish and the least accumulation was found to be in *Streptomyces* sp. isolated from gut of tilapia fish. In marine grouper fish gut, isolated
Streptomyces sp. α-carotene was found.

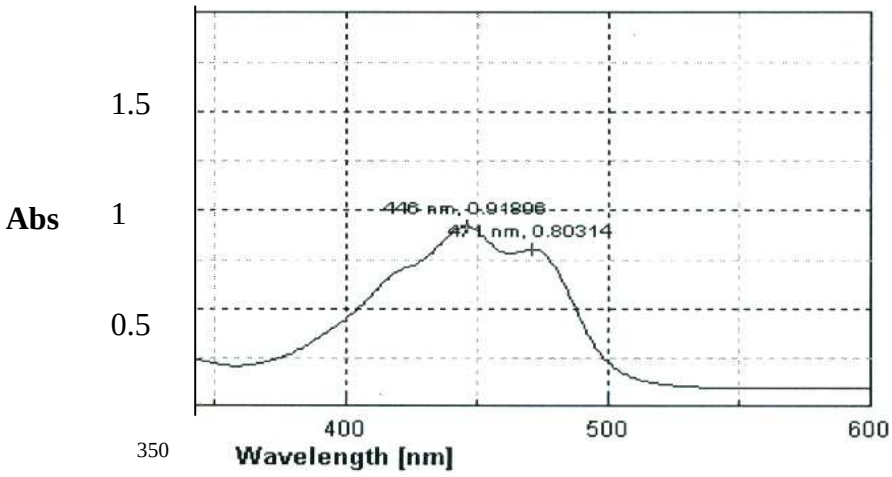
Figure – 1 UV spectral analysis of Carotinoids from selected strains of *Streptomyces* sp

(a)



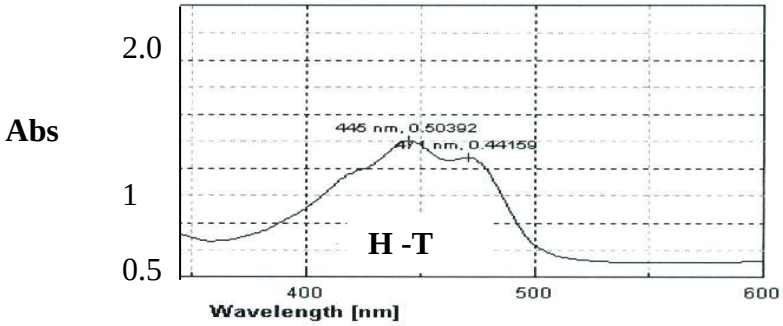
T-T

(b)



E-T

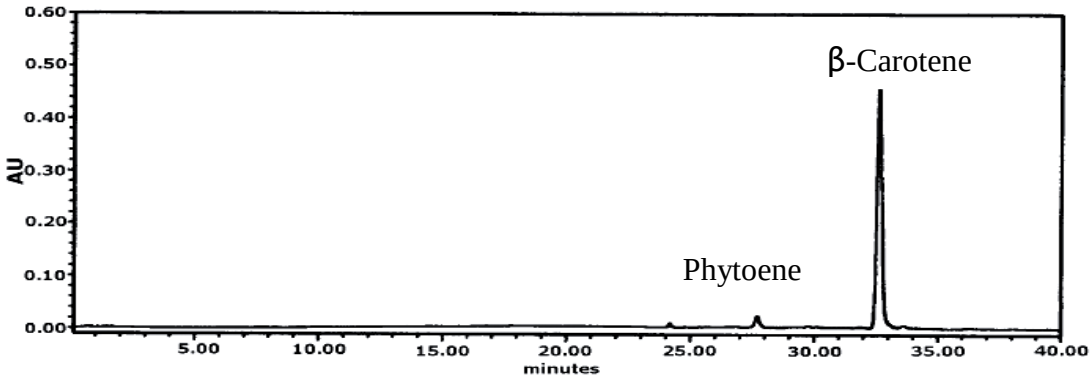
c)



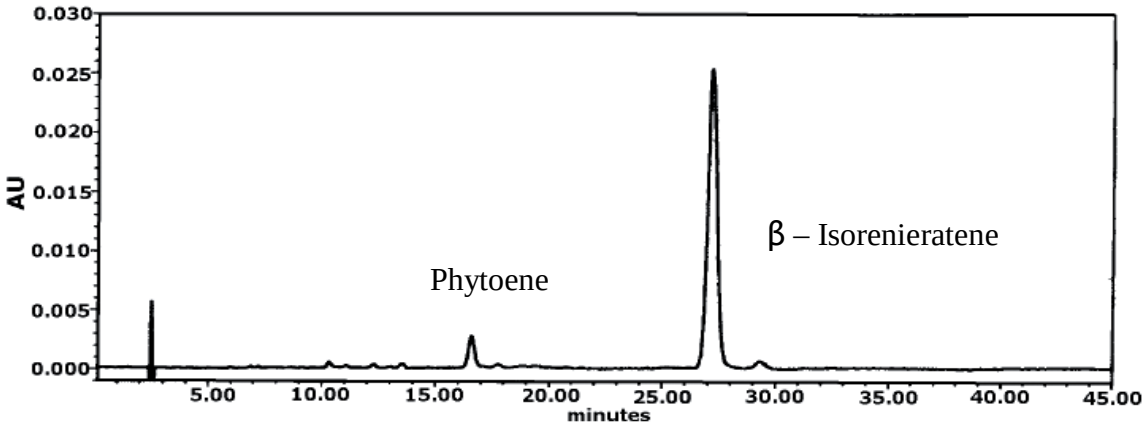
(a) - T T (Tilapia tissue), (b) - **E T**- Etroplus tissue, (c) - **H T**- Hymavathy tissue.

As far as HPLC analysis is concerned it clearly proved the presence of these carotenoids which were earlier identified through UV spectrometer and TLC Fig 2 a,b,c , Table 1. In contrast of *S. mediolani* where 3-hydroxy and 3,3P-dihydroxy derivatives of isorenieratene are formed (17), the carotenogenic pathway of *Streptomyces* sp. ends with the synthesis of isorenieratene. The carotenoids include Phytoene, Phytofluene, α-Carotene, β-Carotene. In *Streptomyces* sp. carotenoid production is a widespread metabolic activity, which occurs in a constitutive, light-dependent, or cryptic manner[9]. This implies the presence of a certain diversity in the molecular mechanisms of carotenoid production in this group of bacteria.

Fig 2 HPLC analysis of Carotenoids from selected strains of *Streptomyces* sp

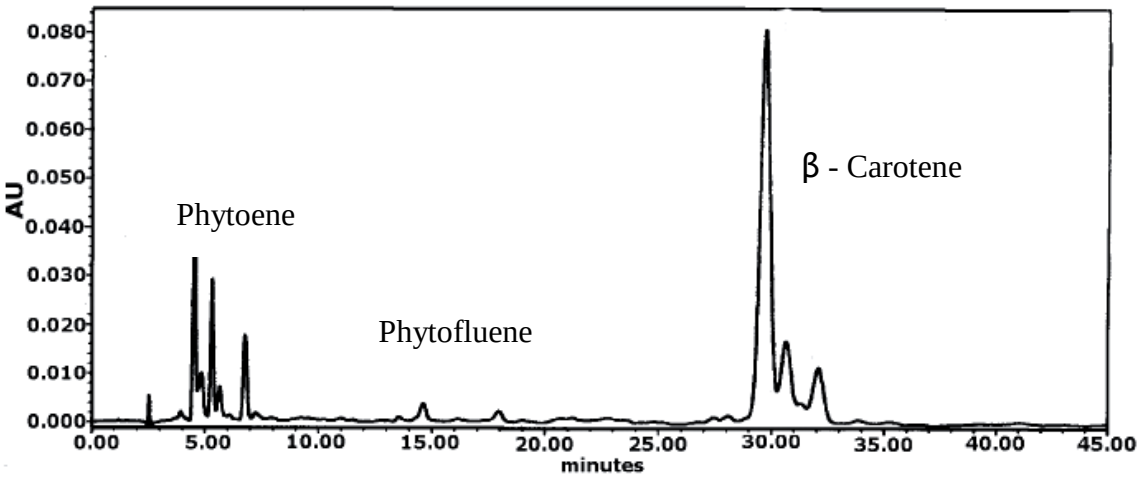


(a)



(b)

(c)



AQB-H

(a) T – Tilapia, (b) E – Etroples (c) H – Hymavathy Pond.

Table 1-Thin layer chromatography analysis of selected strains of *Streptomyces sp.*

Si no	Samples	Rf values	Carotenoids
1	**AQB-T**	0.99 0.93	Phytoene, β-Carotene
2	**AQB-E**	0.99 0.95	Phytoene, β–Isorenieratene
3	**AQB-H**	0.99 0.96	Phytoene, β - Carotene

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. 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.

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