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STUDIES ON EFFICACY OF MARINE BACTERIUM *SALINICOCCUS ROSEUS* PIGMENT FOR THEIR BIOACTIVE POTENTIAL

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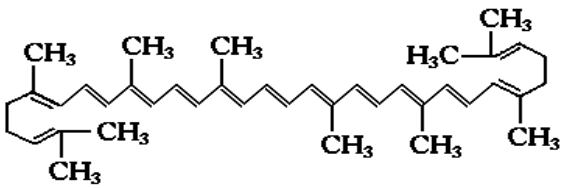
ABSTRACT
The marine*Salinicoccus roseus* sourced from saltpan region of Mukkani, Tuiticorin District, has subjected for their ability to produce lycopene and which was used for the screening of anti-oxidant, anti cancer and anti-diabetic activity. The orange redcoloured colony produced on the Zobell marine agar medium indicates that the organism has ability to produce the pigment lycopene. The biochemical analysis and phylogenetic analysis showed that the strain was *Salinicoccus roseus.* An orange colour spot with R_f value of 0.41 observed for lycopene through thin layer chromatography. The UV- spectral analysis showed that the maximum absorption at 480nm which indicates the lycopene pigment. The HPLC analysis showed, the retention time at 2.88, it confirmed the lycopene pigment. The FTIR analysis revealed that the data for the functional groups of the lycopene pigment. *Salinicoccus roseus* lycopene pigment has better DPPH scavenging antioxidant activity with the IC_{50} value of 250µg/ml. *Salinicoccus roseus* also excert anticancer activity with the IC_{50} value of 250µg and only 47% cell viability was observed. Lycopene pigment extracted from *Salinicoccus roseus* has exerted better antidiabetic activity in *invitro* analysis, α-amylase inhibitory activity was noticed at higher concentration of lycopene pigment.

KEYWORDS: *Salinicoccus roseus*, Anticancer activity, Lycopene, Anti oxidant activity.

INTRODUCTION
Halophiles have a worldwide distribution and have been isolated from a wide variety of habitat, including areas of both low and high salt concentrations. Typical sites of halophilic isolation have included unpurified salt crystals, saline soils, saltern ponds, saline lakes, deserts, oceans and salted hides or foods.[1] Organisms that inhabit these saline environments are called halophilies. The two groups of organisms that are normal inhabitants of these habitats are: i) Extremely halophilic aerobic archaea; members of the family Halobacteriaceae, which includes several species grouped in 15 different genera. ii) Moderately halophilic bacteria, that grow best at lower salinities than halobacteria and are predominant organisms in environments with intermediate salinities.[2] Many moderate halophiles produce orange or pink colonies, probably due to the production of carotenoids as a protective mechanisms against photooxidation processes.

The genus *Salinicoccus* in the family *Staphylococcaceae* is defined as moderately halophilic, aerobic, Gram-positive, non-motile, non-sporulating, and heterotrophic cocci.[3] Many moderate halophiles produce orange or pink colonies, probably due to the production of carotenoids as a protective mechanisms against photooxidation processes.



Structure of Lycopene

Lycopene has attracted attention for nearly 50 years for its biochemical and physiochemical properties. Since that time, epidemiological, *invitro* and *invivo* animal and human experiments have provided support for lycopene's anti-oxidant health benefits and its potential to reduce the risk of several cancers and cardiovascular diseases. The effect of lycopene decreased, serum lipid peroxidation, decreased serum LDL oxidation and decreased protein oxidation.[4] The present study was carried out to realizing the characterization of lycopen pigment from *Salinicoccus roseus* and also to evaluate its bioactive potential.

MATERIALS AND METHODS

Collection of marine soil sample

Saltpan soil sediment sample was collected from saltpan region Mukkani of Tuticorin District, the marine bacterium *Salinicoccus roseus* was isolated by serially dilute the sediment sample and plated on Zobell marine nutrient agar medium.

Identification and characterization of the sample

The pure culture of *Salinicoccus* sp was identified based on their morphology and colony characteristics. The organisms were maintained on Halophilic agar medium as well as Zobell marine agar medium and stored at 4^0C.[5] DNA was extracted from the marine bacterial isolate and it was subjected for 16s rRNA sequencing.[6] Then the pure culture of *Salinicoccus roseus* was selected and screened for lycopene pigment production in salty nutrient medium.

Extraction of pigment produced by *Salinicoccus roseus*

For the extraction of bacterial pigment, 100ml of marine broth cultures of *Salinicoccus roseus* were centrifuged at 8,000 rpm for 10 min at 4^0C. The supernatant was separated and methanol was added to the pellet and followed by further centrifugation at 10,000 rpm for 10 min at 4^0C. Then the pellet was transferred into watch glass and allowed it to dry until the solvent evaporated and orange red colored crystals appeared.

Spectroscopic analysis

Pigment extracted with methanol solvent were subjected for spectroscopy and analysed between 300nm and 500nm using UV Spectrophotometer and it shows individual peaks for respective carotenoids with their optical densities.

TLC analysis of carotenoids

Qualitative analysis of Lycopene carotenoid in the experimental sample was carried out by using Thin Layer Chromatograpy (TLC). 3µl condensed lycopene carotenoid samples were spotted on the silica gel TLC plates and that was placed in a presaturated TLC chamber contains mobile phase. (5% Methanol/Toluene in the ratio of 95.5v/v). Then the chromatogram was developed and relative R_f values were calculated.

HPLC and FTIR analysis

The band separate through TLC was spotted out and redissolved in methanol and was subjected for HPLC and FTIR analysis then the results were recorded.

Antioxidant activity determination by DPPH Assay:

Free radical scavenging activity of extracted lycopene pigment was measured by DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay.[7] The extracted pigment was dissolved in methanol to give concentration from 0.2-0.8µL/mL. Then 1ml of the extracted pigment and added into 2ml of methanolic solution of 10µM DPPH. For control reaction, extracted pigment was replaced with 100% methanol. The mixture was incubated for 2hours in dark at ambient temperature. Finally, the absorbance was measured at 517nm using an ultra UV spectrophotometer (Agilent 8453 UV-Spectrocopy system, Germeny). For positive standard control Ascorbic acid was used. Antioxidant activity was calculated using the following equation:

$$\text{DPPH Scavenging effect (\%)} = [A_0\text{-}A_1/ A_0] \times 100$$

Where A_0 is the absorbance of the control reaction (containing all reagents with methonal) and A_1 is the absorbance of the extracted sample in the DPPH solution. The %DPPH radical inhibition was plotted against the sample concentrations for calculation of the IC_{50} value. **α- Amylase Inhibitory Assay**

α- amylase inhibitory activity was determined by measuring the amount of maltose released using colorimetric method with a slight modification.[8] The extracted pigment was dissolved in methanol to give concentrations from 100 to 500µg/ml. The enzyme α-amylase solution was prepared by mixing of α- amylase in 100ml of 40mmol/L phosphate buffer, pH 6.9. Positive control, acarbose was obtained by dissolving in phosphate buffer. The assays were conducted by mixing of 50µL of α- amylase and 50µL of pigment extract then the mixture was incubated at 25^0C for 30min. After that, 100µL of starch solution (0.5% w/v) was added and 100µL of the color reagent (20mL of 96mM 3,5 dinitrosalicylic acid, 5.31M sodium potassium tartarate in 2M sodium hydroxide (8mL) and 12mL of double deionized distilled (DDD water) was added and incubated at 85^0C for 15min. The mixture was then cooled and 900µL of DDD water was added. The final mixture was measured at 540nm. α- amylase activity was assessed by the formula:

$$\text{Inhibition (\%)} = 100 \times (\Delta A_{control} - \Delta A_{sample}) / \Delta A_{control}$$

$$\Delta A_{control} = A_{Test1} - A_{Blank1}$$

$$\Delta A_{sample} = A_{Test2} - A_{Blank2}$$

Where A_{Test1} and A_{Test2} are defined as the absorbance of methanol and sample with α- amylase. A_{Blank1} and A_{Blank1} are defined as the absorbance of methanol and sample without α- amylase enzyme.

Anticancer activity by Cytotoxicity assay

The human laryngeal cancer cell line (Hep-2) cell line was maintained of CLRI Department of Biomolecular engineering, Chennai, India. Cells were grown as monolayer culture in MEM medium and incubated at 37°C in a 5% of CO_2 atmosphere. Hep-2 and VERO cells (100µl) were seeded in 96 well plates at a concentration of $5X10^3$ cells/ml for 24 hrs. After the incubation the culture medium was replaced with 100ml serum free medium containing various concentrations of *Salinicocus roseus* extracts at 24 hrs and 48 hrs. After that, the medium was refreshed with 100µl of serum free medium (MEM) and 20µl of MTT (5 mg/ml of (3, 4, 5-dimethylthiazol- 2yl)-2,5-diphenyltetrazoliumbromide) was added. The microtiter plates were incubated for three

hours in dark. The developed color was measured with ELISA reader at 570 nm. Triplicates were maintained for each treatment. IC_{50} values were determined by calculating the % of viability:

$$\% \text{ of viability} = \frac{\text{Mean Test OD}}{\text{Mean OD of Control}} \times 100$$

RESULTS AND DISCUSSION

Characterization of the sample

The marine soil sample was collected from saltpan region of Mukkani, Tuticorin District. The isolated marine bacterial culture was identified as *Salinicoccus sp*due to its capability to produce the red orange colored colony in the Zobell marine medium.

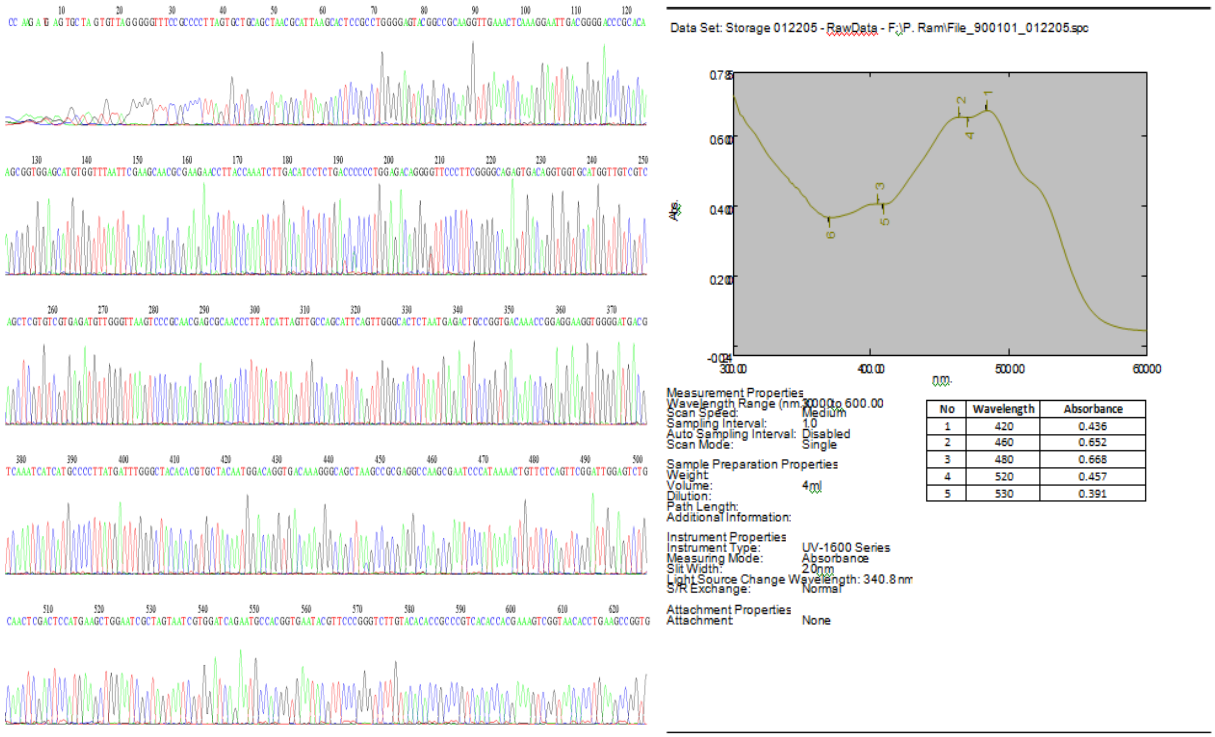
The isolated culture showed the morphological characteristics as Gram positive, non-motile, cocci shaped cells with smooth surface. The sequencing results confirmed that the sample was *Salinicoccus roseus* which was showed in the following findings. ("Fig 1").

Fig 1: Isolate of *Salinicoccussp* in Zobell marine nutrient medium

Thin layer chromatography analysis of lycopene from *Salinicoccus roseus* revealed that the orange color spot was observed after air drying of developed plate. The R_f value of the obtained lycopene was 0.41.

The UV spectral analysis of lycopene pigment showing the maximum absorption at 480nm ("Fig 2"). The HPLC data for the present study showed that the peak at various retention times. The peak obtained at the retention time of 3.653 indicates the presence of lycopene pigment ("Fig 3").

The FTIR spectral analysis of the Lycopene ("Fig 4") reveals that the FTIR data showed the peak values at 2924.18, 2854.74, 1743.71 and 1026.16. Each of the values represented the functional group of lycopene having the molecular formula of $C_{40}H_{56}$.



Result: *Salinicoccus roseus*

Fig 2: Sequence Results

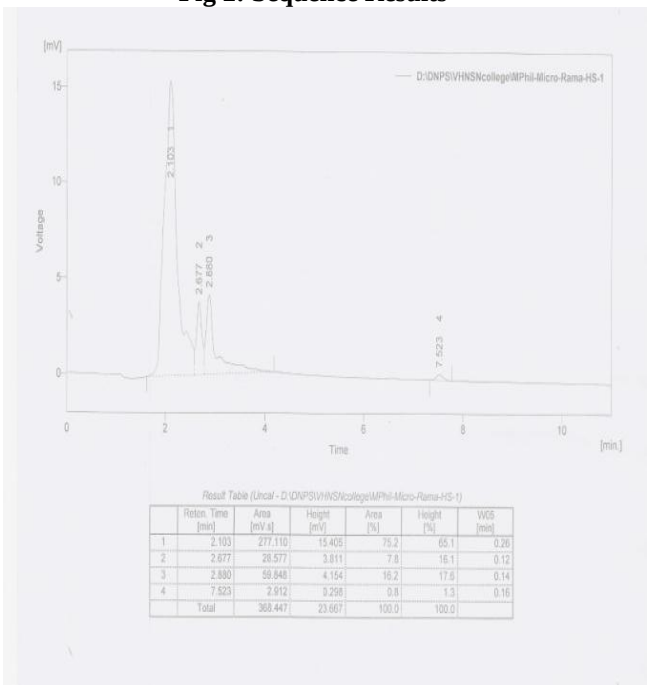


Fig 3: UV Spectral analysis

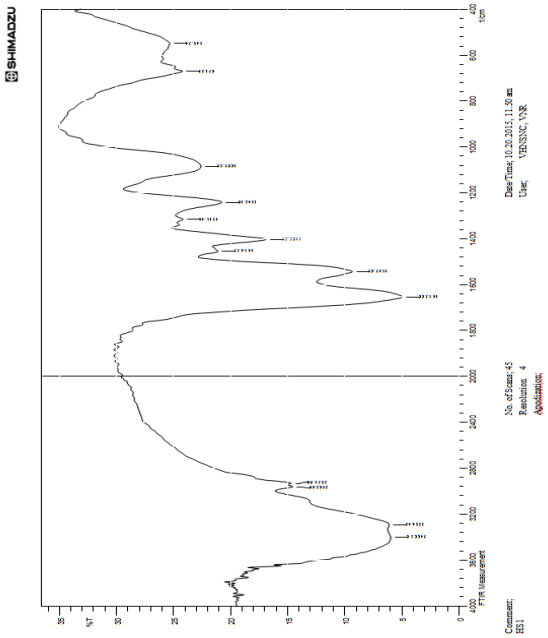


Fig 4: HPLC Results

Fig 5: FTIR Results

Anti-oxidant activity of lycopene pigment of *Salinicoccus sp*

Evalution of the antioxidant activity of the *Salinicoccus roseus* lycopene pigment exhibited antioxidant activity against DPPH. The concentration required to inhibit 50% of free radicals is as illustrated in the ("Fig 6"). There was a linear increase in the antioxidant activity with increase in the lycopene concentration. Strong inhibition of these radicals which could in-turn inhibit the patho-physiological events, including inflammation, cellular and systemic disorder.[9]

Anticancer activity of lycopene pigment of *Salinicoccus* sp

Cytotoxicity assay was done with lycopene pigment extracted from marine *Salinicoccus roseus* against human laryngeal cancer cell line (HEP-2) by performing MTT assay. IC_{50} value of lycopene pigment was calculated. Among the different concentration used for anticancer assay 250µg concentration of lycopene pigment inhibit 53% cell viability which was considered as IC_{50} value. Other concentration, 500µg of lycopene pigment completely inhibit the cell viability. ("Fig 7").

Fig 6: Anti oxidant activity by DPPH method

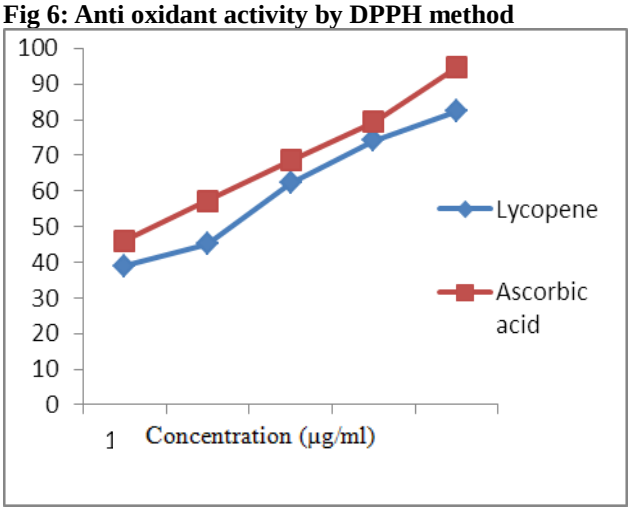


Fig 6: Represents that DPPH scavenging effect of lycopene. Red bar indicates the DPPH scavenging effect of Ascorbic acid as a positive control. Blue bar indicates the DPPH scavenging effect of Lycopene. In X-axis have concentration of compound and Y-axis have DPPH scavenging effect.

Fig 7: Anti-Cancer Activity

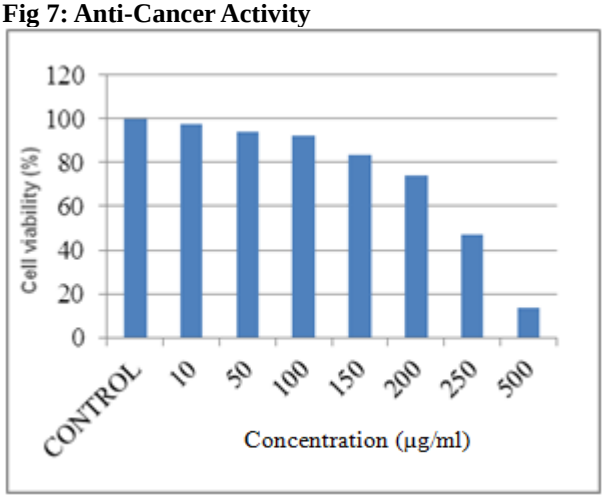


Fig7: Represents that Anticancer activity of lycopene. In X-axis have concentration of *Salinicoccus roseus* lycopene pigment and Y-axis cell viability in percentage at 24hours.

Anti-Obesity Activity

Evaluation of the Anti-obesity activity of the *Salinicoccus roseus* lycopene pigment exhibited α-amylase inhibitory activity. The concentration required to inhibit 50% of α-amylase enzyme was illustrated in the ("Fig 8"). There was a linear increase in the α-amylase inhibitory activity with increase in the lycopene concentration. In our study, acarbose used as a positive control, which also showed a linear increase in the α-amylase inhibitory activity. This result indicates that the lycopene pigment is very potent α- amylase inhibitor in comparison with acarbose.[10] Strong inhibition of these α-amylase enzyme which could not convert glycogen to glucose so that it was control blood glucose level and may be used for the treatment of diabetic patients.

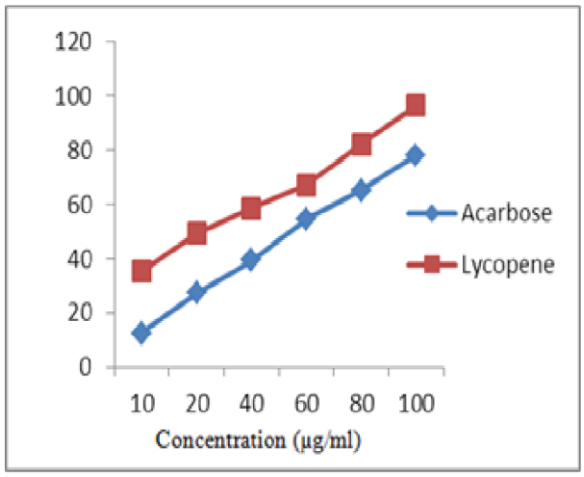


Fig 8: Represents that Anti-Obesity activity of lycopene. Red bar indicates the α-amylase inhibitory activity of Acarbose as a positive control which is a commercial antidiabetic drug. Blue bar indicates the α-amylase inhibitory activity of Lycopene. In X-axis have concentration of compound and Y-axis have α-amylase activity inhibition percentage.

CONCLUSION

Salinicoccus roseus are thus well adapted and are functional members of the marine microbial community moreover the present investigation has evolved that coastel area of Mukkani as a source of marine halophiles. These marine halophile *Salinicoccus roseus* has the potential ability to produce lycopene pigment and this pigment significantly has anti-oxidant, anti-cancer, anti-diabetic activity. Thus it is a potential lead compound which can be considered for development as therapeutic for the treatment of diabetes and cancer.

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