Isolation of astaxanthin from marine yeast and study of its pharmacological activity

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ABSTRACT
The aim of the present study was to isolate and study about the antibacterial activity of astaxanthin from marine yeast. Astaxanthin a carotenoid present in marine yeast and crustaceans posses a wide range of pharmacological activity. Yeast strain was isolated from the marine sediments collected from Cochin, Kerala during the month of August 2012. The samples were collected in sterile plastic covers and brought to the laboratory without exposing to the external environment further. The marine sediment samples were directly inoculated in yeast malt. After effective screening the strains containing astaxanthin was refrigerated for further use. The strains were purified by TLC using petroleum hexane: acetone (3:1). A spectrophotometric assay was carried out by measuring absorbance at 200-500nm. The antibacterial activity was studied on several organisms like Bacillus Subtilis, Salmonella typhi, Staphylococcus aureus and Pseudomonas aeroginosa. The extracts showed excellent antibacterial activity than the standard chloramphenicol. Among this, Pseudomonas aeruginosa showed maximum inhibition.

Key Words: Astaxanthin, marine yeast, yeast malt, Antibacterial activity, fermentation, well diffusion assay.

INTRODUCTION
Astaxanthin (Figure 1), unlike some carotenoids, does not convert to Vitamin-A (retinol) in the human body. Too much Vitamin A is toxic for a human, but astaxanthin is not. However, it is a powerful Antioxidant; it is 10 times more capable than other carotenoids. While astaxanthin is a natural nutritional component, it can be found as a food supplement. The supplement is intended for human, animal, and aquaculture consumption. The commercial production of astaxanthin comes from both natural and synthetic sources (Mortensen and Skibsted, 1997; Texier et al., 1984).

The US Food and Drug Administration (FDA) approved astaxanthin as a food colouring (or colour additive) for specific uses in animal and fish foods. The European Union (actually European Commission) considers it food dye within the E number system. Astaxanthin pronounced as (as-tuh-zan’-thin) is a carotenoid. It belongs to a larger class of phytochemicals known as terpenes. It is classified as a xanthophyll, which means "yellow leaves". Like many carotenoids, it is a colorful, fat/oil-soluble pigment. Astaxanthin can be found in microalgae, yeast, salmon, trout, krill, shrimp, crayfish, crustaceans, and the feathers of some birds. Professor Basil Weedon was the first to map the structures of astaxanthin. The study of the pharmacological activity of astaxanthin is vast and wide. The aim and scope of the present research work is to study the anti bacterial activity of astaxanthin (Moren et al., 2002; Lorenz and Cysewski, 2000)

MATERIALS AND METHODS
Sample collection
Yeast strain was isolated from the marine sediments collected from Cochin, Kerala during the month of August 2012. The samples were collected in sterile plastic covers and brought to the laboratory without exposing to the external environment further.
Isolation of marine yeast
The marine sediment samples were directly inoculated into Yeast malt (YM) broth fortified with chloramphenicol to prevent bacterial contamination and incubated at 24±1°C for 3 to 5 days. The colonies developed were purified by inoculating each isolates into fresh Yeast malt Agar medium (Table 1). The cultures were screened for astaxanthin production and the efficient strain producing astaxanthin was periodically subcultured and refrigerated for further use.

Aspergillus (crude saccharidase) preparation
20g bran, 5g flour and 20ml water were taken and mixed into 500ml Erlenmeyer flask, sterilized at 121°C for 20 min. Then the solution was cooled to 30°C and inoculated Aspergillus niger AS. 3.278 and Aspergillus flavus AS. 3.800, respectively under aseptic condition. They were cultured at 30°C for 12 hrs. Closing the flask till mycelium grown over shake flask and forming into shape of pie in 24 h, continue culturing till substrate were fully grown in spore. The overall procedure would take 72 h or so.

Saccharification of Cassava Residues
100g cassava residues was taken into 1000ml triangular flask, 700ml water was added and sterilized at 121°C for 20 min. After sterilization, cultured aspergillus preparation was added (3g AS. 3.800 and 7g AS.3.2783) and saccharificated at 58°C for 5 hrs.

Nitrogen sources medium
Saccharified cassava residues were filtered for solution. Sugar degree was adjusted to 30 g/L through glucose, yeast extract of 3g/L, (NH4)2SO4 of 2g/L, KH2PO4 of 1.5g/L, and MgSO4 of 0.5g/L were added and transferred into 100ml/500ml triangular flask, respectively and sterilized at 121°C for 20 min.

Analytical methods
Pigment extraction
The extraction and analysis of Astaxanthin content in the yeast cells followed the methods as described by Du et al. (2008). Yeast biomass was separated from the liquid medium by centrifuging and rinsed twice with double distilled water. The yeast was disrupted with dimethyl sulphoxide (DMSO) and then extracted with Acetone. The amount of Astaxanthin was calculated from the absorbance measured at 480nm multiplying an extinction coefficient of 2150.

Dry weight determination
Yeast biomass was separated from the liquid medium by centrifuging and rinsed twice with double distilled water, and then dried at 105°C overnight to constant weight, yielding the dry weight.

Study of antibacterial activity
Agar diffusion assay is used widely to determine the anti-bacterial activity of Leaf extract. The technique works well with defined inhibitors (Hewit and Vincent, 1989). Nutrient agar prepared was poured in the Petri dish. 24 hours growing culture (Salmonella typhi; Pseudomonas aeruginosa; Bacillus subtilis and Staphylococcus aureus) were swabbed on it. The wells (10mm diameter) were made by using cork borer. The different concentrations of the crude extract were loaded in the wells. The plates were then incubated at 37°C for 24 hours. The inhibition diameter was measured (Figure 2).
Astaxanthin is effective against gram positive and gram negative bacteria when compared with standard chloramphenicol. However the experiments have to be tried by using different solvents.

**REFERENCES**


**RESULTS AND DISCUSSION**

Table 1 shows the composition of medium of extract. Table 2 shows the diameter of zone of inhibition of extracts on various microorganisms when compared to the standard. Figure 2 shows the well diffusion assay zones produced by standards and various microorganisms. The antibacterial activity of the extract was studied by using well diffusion method Salmonella typhi produced 20mm diameter for zone of inhibition, Pseudomonas aeruginosa 24 mm, Bacillus subtilis 18 mm. Staphylococcus aureus 16mm. The extract showed excellent antibacterial activity than the standard chloramphenicol. Among this, Pseudomonas aeruginosa (Figure 2, Table 2) showed maximum inhibition.

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Microorganism</th>
<th>Extract</th>
<th>Standard</th>
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<tbody>
<tr>
<td>1</td>
<td>Salmonella typhi</td>
<td>20</td>
<td>24.5</td>
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<tr>
<td>2</td>
<td>Pseudomonas aeruginosa</td>
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</tr>
<tr>
<td>3</td>
<td>Bacillus subtilis</td>
<td>18</td>
<td>24</td>
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<tr>
<td>4</td>
<td>Staphylococcus aureus</td>
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<td>23</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Astaxanthin is effective against gram positive and gram negative bacteria when compared with standard chloramphenicol. However the experiments have to be tried by using different solvents.