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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 aeroginasa* 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)

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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.

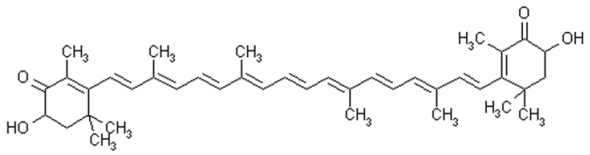

Figure 1: Structure of Astaxanthin.

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 saccharified 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, KH_2PO_4 of 1.5g/L, $MgSO_4$ of 0.5g/L, were added. 5g/L of peptone, yeast extract, beef extract, KNO_3, $(NH_4)_2SO_4$ and NH_4NO_3 were added. Then, it was transferred into 100ml/500ml triangular flask, and sterilized at 121°C for 20 min.

Growth medium

Saccharified cassava residues were filtered for solution. Sugar degree was adjusted to 30 g/L through glucose, yeast extract of 3g/L, $(NH_4)_2SO_4$ of 2g/L, KH_2PO_4 of 1.5g/L, and $MgSO_4$ of 0.5g/L were added and transferred into 100ml/500ml triangular flask, respectively and sterilized at 121°C for 20 min.

Table 1: Composition of the medium of extraction.

Ingredients	Amount in grams/litre	
	Yeast malt	Yeast malt agar
Peptic digest of animal tissue	5.0	5.0
Yeast extract	3.0	3.0
Malt extract	3.0	3.0
Dextrose	10.0	10.0
Agar	-	20.0
Final pH	6.2±0.2	-

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).

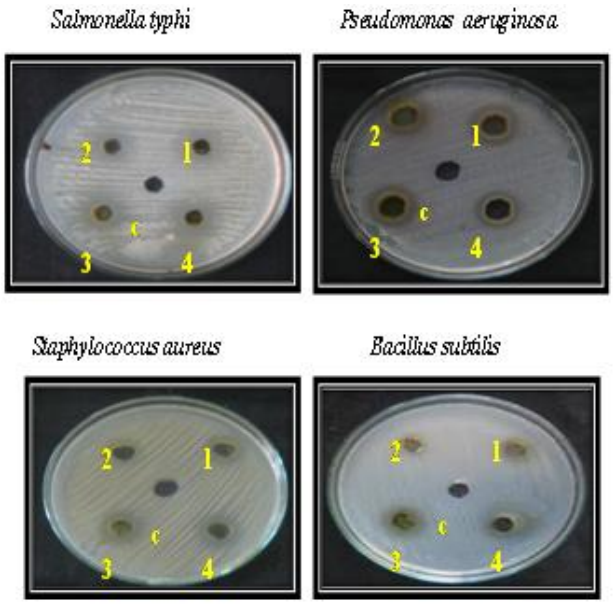

Figure 2: Zone of Inhibition (C-Control; 1-25µg; 2-50µg; 3-75µg; 4-100µg).

Table 2: Diameter of zone of inhibition of extracts on various microorganisms.

Sl No.	Microorganism	Extract	Standard
1	*Salmonella typhi*	20	24.5
2	*Pseudomonas aeruginosa*	22	21
3	*Bacillus subtilis*	18	24
4	*Staphylococcus aureus*	16	23

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, *Pseudogonas aeroginasa* 24 mm, *Bacillus subtilis* 18 mm. *Staphylococcus aureus* 16mm. The extract showed excellent antibacterial activity than the standard chloramphenicol. Among this, *Pseudomonas aeroginasa* (Figure 2, Table 2) showed maximum inhibition.

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.

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