Introduction

Astaxanthin (Figure 1) is the main carotenoid pigment found in microorganisms (the yeast Phaffia rhodozyma and the algae Haematococcus pluvialis), aquatic animals and many of the seafood including salmon, trout, red sea bream, shrimp, lobster and fish eggs. It is also present in birds such as flamingoes and quails. Availability of astaxanthin has also been reported in mangrove flora, under different tidal conditions.

Figure 1 Structure of Astaxanthin.

The mangroves of Indian Sundarbans, being a reservoir of several bioactive substances may serve as future source of astaxanthin, although very few works have been carried out on this particular subject. Astaxanthin has several essential biological functions in animals and is known as a free radical quencher and powerful antioxidant reagent. Industrially, astaxanthin has been commercially exploited as a pigmentation source and feed supplement for marine fish aquaculture and poultry. It is evident, therefore, that astaxanthin is a molecule with potential to the pharmaceutical, food, cosmetic and medical fields.

The industrial production of astaxanthin has been successful in many parts of the world through series of chemical reactions, but the synthetic type is not similar to that found in nature as its stability and activity is lower than that of natural product. Therefore attention has been diverted for searching the natural source of astaxanthin. The deltaic complex of Indian Sundarbans at the apex of the Bay of Bengal (21°13′ to 22°40′ N latitude and 88°03′ to 89°07′ E longitude) sustains 34 species of true mangroves, which are specialized floral community found at the land-sea interface in the estuarine and coastal ecosystems (Figure 2).

These plants are characterized with the presence of pneumatophores, prop roots, still roots and viviparous germination and are exposed to tidal inundations twice in every 24 hours in this part of the Indian subcontinent. The important ecological requisites for the growth of these plants are water salinity (ranging from 2% to 30%), pH (around 8.00), tidal inundation and clayey-silty soil substratum typical for deltaic complex. Considering the immense ecological and economic benefits of these plants, the Govt. of India (Ministry of Environment and Forests) has taken up several programmes of afforestation and nursery development in and around Indian Sundarbans and hence proper back-up of raw materials exists in this region that can serve as natural source of astaxanthin.

Materials and methods

Sagar Island is the largest island of Indian Sundarbans (located between 21°31′ N to 21°53′ N latitude and 88°02′ E to 88°15′ E meridians) with an approximate area of 145sq.km. The entire network of the present programme consists of the sampling of five dominant mangrove species from the northern and southern tips of Sagar Island during April, 2016. These two sampling stations are drastically

Abstract

The astaxanthin level in the leaves of five dominant mangrove species, sampled from two different stations of Sager Island in western Indian Sundarbans region was studied during April, 2016. The two sampling stations selected at the northern and southern tips of the island exhibited drastic variation with respect to aquatic salinity, although other parameters like surface water temperature, pH, D.O. and nutrients were more or less uniform. Relatively higher astaxanthin level was observed in the high saline zone (southern tip) of the island, which indicates the regulation of astaxanthin level in the mangrove plants by ambient aquatic salinity. The inundated leaves of the selected mangrove plants showed more astaxanthin level in comparison to the exposed ones. The astaxanthin content of the mangrove leaves was in the order Heritiera fomes > Avicennia alba > Avicennia marina > Avicennia officinalis > Sonneratia apetala in both the sampling stations which points towards the species specificity of the carotenoid pigment.

Keywords: mangroves, astaxanthin, Indian sundarbans, tidal influx, salinity gradient

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different from each other with respect to salinity as the northern tip of the island lies within the Hugli estuarine stretch, whereas the southern tip is located at the confluence of the river Hugli and the Bay of Bengal. Leaves of the selected species were collected during the low tide period from two different portions (submerged lower zone and exposed upper zone) of the same plant. The lower region of the tree is inundated during the high tide condition and the upper region of the same plant remains unexposed to tidal water. The collected leaves were thoroughly washed with ambient water followed by deionized water and oven dried at 110°C overnight. The estimation of carotenoid were separately carried out for each species through spectrophotometric method after extraction with DMSO and acetone and finally the carotenoid value was converted to astaxanthin percent as per the expression: Percent astaxanthin=Carotenoids (mg) extracted/ sample weight (mg)×80.

Water samples of both the stations were also collected simultaneously for analyzing the physico-chemical variables (surface water temperature, salinity, pH, DO, nitrate, phosphate and silicate) as per the standard methods.12

Results and discussion

The astaxanthin level in the selected mangrove species exhibits significant spatial variation (Table 1).

| Station       | Mangrove species       | Astaxanthin content (mg/kg) | Submerged | Exposed |
|---------------|------------------------|-----------------------------|-----------|---------|
| Kachuberia    | Avicennia alba          | 485.08                      | 313.02    |         |
| (salinity range: 1‰ to 9‰) | Avicennia marina        | 409.66                      | 298.73    |         |
|               | Avicennia officinalis   | 388.02                      | 267.44    |         |
|               | Sonneratia apetala     | 156.14                      | 94.32     |         |
|               | Heritiera fomes        | 619.20                      | 386.25    |         |
|               | Avicennia alba          | 513.95                      | 353.20    |         |
| Sagar south   | Avicennia marina        | 449.36                      | 340.13    |         |
| (salinity range: 12‰ to 28‰) | Avicennia officinalis  | 420.10                      | 310.41    |         |
|               | Sonneratia apetala     | 225.21                      | 130.42    |         |
|               | Heritiera fomes        | 680.22                      | 391.02    |         |

Table 1: Astaxanthin content in five mangrove species collected from two different stations of Sagar Island, Sundarbans during April 2016

The two sampling stations in the present programme are drastically different with respect to surface water salinity although other parameters are more or less uniform (Table 2).

Table 2: Physico-chemical variables of water samples collected from two different stations of Sagar Island, Sundarbans during April, 2016

| Parameters                      | Northern tip | Southern tip |
|---------------------------------|--------------|--------------|
| Surface water temp (°C)         | 33.0±0.02    | 32.2±0.01    |
| pH                              | 8.0±0.01     | 8.2±0.01     |
| Salinity (psu)                  | 8.5±0.31     | 27.23±0.44   |
| DO (mg/l)                       | 5.82±0.23    | 6.01±0.19    |
| NO₃ (µgat/l)                    | 15.32±0.31   | 16.2±0.39    |
| PO₄ (µgat/l)                    | 1.72±0.05    | 1.45±0.04    |
| SiO₄ (µgat/l)                   | 85.90±1.07   | 91.24±0.98   |

The proximity of the southern tip of Sagar Island to Bay of Bengal may be attributed to high aquatic salinity in this zone. The relatively higher astaxanthin level in the plant parts collected from the southern tip of Sagar Island may be related to stress posed by high aquatic salinity. Similar variations of the pigment level with geographic location have been pointed out by several workers.13 In both the sampling stations, a uniformity was observed with respect to astaxanthin level of the mangrove leaves and it is in the order Heritiera fomes > Avicennia alba > Avicennia marina > Avicennia officinalis > Sonneratia apetala (Table 1). Astaxanthin occurs in several different forms and can be classified into stereoisomer’s, geometric isomers and free or esterified forms. All these forms are found in various natural sources e.g., the predominant stereoisomers of astaxanthin found in the Antarctic krill Euphausia superba is 3R,3R and the majority of this is esterified.14-16 The relative distribution of esters and optical isomers in some organisms is shown in Table 3. The level of astaxanthin detected in the mangrove floral species of Indian Sundarbans is less than the existing natural mega-reservoir of astaxanthin like Phaffia rhodozyma,17 Whyte18 and Haematococcus pluvialis.21 However considering the huge biomass of mangrove leaves in and around the area of Sundarban Biosphere Reserve (SBR), the standing stock of the carotenoid pigment has high probability to reach the point of compensation (requirement), provided few back-up nurseries in the region for extraction of bioactive substances are maintained.

The relatively higher astaxanthin content in the submerged leaves of mangrove plants confirms the synthesis of the pigment under stress condition. However, more studies are needed to establish the role of tidal influx and subsequent salinity fluctuation of the ambient aquatic phase on astaxanthin level in the mangrove floral parts. The present data may serve as baseline information on the regulatory role of tidal submergence on astaxanthin level in the estuarine and coastal vegetation. The enhancement of astaxanthin production under stressed condition of organisms is a matter of interest and several researches are still being undertaken to pinpoint the reaction pathway of astaxanthin production by inducing stress of varied nature. Many yeasts have been described with an increase ability to produce carotenoids when they grow under unfavorable environment.22 The enhancement of the accumulation of astaxanthin in cysts of Haematococcus pluvialis

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under salt stress conditions has been reported both in the dark and
light by several workers.\textsuperscript{23-31} The present study has pointed higher
astaxanthin level in those leaves of the mangroves that are inundated
for 10 to 12 hours by tidal waters (ambient water salinity during the
period of investigation was 8.50\% in the northern tip of Sagar Island
and 27.23\% in the southern tip of Sagar island) thus exposed to stress
caused by aquatic salinity. The steep enhancement of astaxanthin
level in the inundated Sundari leaves \textit{(Heritiera fomes)} irrespective
of the sampling stations (space) clearly reflects the highest degree of
stress posed by water salinity on this species. \textit{Heritiera fomes}, being
fresh water loving mangrove species cannot tolerate high salinity\textsuperscript{22}
and hence acceleration of astaxanthin production may probably be
a part of its adaptation to cope with the stenohaline condition of
coastal and estuarine environment that becomes acute during high
tide. The astaxanthin level of mangrove flora is thus a function of its
physiological system, which is extremely species specific.

| Astaxanthin                      | Free | Diester | Monoester | Ratio optical isomers |
|---------------------------------|------|---------|-----------|-----------------------|
| *Euphausia superba* (Antarctic krill) | 5    | 64      | 31        | 9:21:70               |
| *Thysanoessa inermis* (Antarctic krill) | 4    | 61      | 35        | 55:07:38              |
| *Calanus finmarchicus* (Marine copepod) | 11   | 46      | 43        | 83:03:14              |
| *Acanthephyra purpurea* (Deep sea shrimp) | 20   | 43      | 37        | 20:44:15              |
| *Cancer pagurus shell* (Edible crab shell) | 58   | 22      | 13        | 20:24:56              |
| *Phaffia rhodozyma* (Red yeast) | 100  |         |           | 98:+:+                |
| *Haematococcus pluvialis* (Alga) | 5    | 59      | 22        | 4:8:88                |

*The free and esterified astaxanthin and ratio of optical isomers is based on refs 15-18.

In both plant and animal kingdoms, the protective role of
astaxanthin to tide over unfavorable environmental conditions is very
prominent. This carotenoid pigment plays many important functions
in fishes, crustaceans and several aquatic animals like improved
protection against oxidation and photo-oxidation, reproduction
and development, immune response, resistance to diseases and
communication system. Recent research shows that astaxanthin acts
like a vitamin for salmon. Now-a-day astaxanthin is used as a source
of pigmentation for fish in aquaculture and for eggs in the poultry
industry.\textsuperscript{35} It also recognized as having a higher antioxidant activity
than other carotenoids,\textsuperscript{34,35} Kobayashi\textsuperscript{36} and hence has a great role to
play in the sector of aqua cultural feed and poultry feed production.
The present research has immense relevance in the framework of
Indian Sundarbans as several small scale mangrove based astaxanthin
industries like cattle feed, fish feed, poultry feed units can be set up
involving the local people.

This will not only improve the quality of products, but can bind
substantial number of island dwellers in the matrix of alternative
livelihood schemes, who otherwise are engaged in illegal intrusion
in the mangrove forest for exploitation of natural resources, poaching
of wild animals, honey collection, prawn seed collection and several
anti-conservation related activities.

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transportation and low cost fly ash based structures and propagating
the same through special Franchise Model developed by Prof. Sujoy
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**Conflict of interest**

The author declares no conflict of interest.

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