Introduction

Colour is a vital constituent and is probably one of the first characteristics perceived by the senses. Natural colours are generally extracted from fruits, vegetables, roots & microorganisms and are often called bio-colours because of their biological origin [1]. There is an increasing demand for natural colors in the food, pharmaceutical, cosmetics, textile, printing and dye industry. In recent year’s utilization of natural pigments in food stuff, dye stuff, cosmetics & pharmaceutical manufacturing process has been increased [2].

Colourants are added to many foods to make them appear healthier and more appealing; reinforcing the fact that colour plays an important role in consumer choice [3]. With the increasing awareness of toxicity of synthetic colours, a demand for pigments from natural sources has been increased [4]. Among that natural pigment from microbial sources are potentially good alternative ones to synthetic pigments. Natural pigments can be obtained from two major sources of plants [5] and microorganisms [6]. Microbial pigments are a promising alternative to other colour additives extracted from vegetables (or) animals because they are considered as natural, pose no seasonal production problems and show high productivity.

Microorganisms produce various pigments like carotenoids, melanin, flavins, monascins, violacin and indigo [7]. Carotenoids are a group of bioactive compounds and are responsible for bright yellow, orange, red pigments of various plants, microorganisms and animals [8] and are widely distributed in nature [9]. These pigments have an important function to act as protective agents against oxidative damage [10]. Recently carotenoids have attracted greater attention due to the beneficial role on human health. Carotenoids can inhibit various types of cancer and it enhanced the immune response [11]. It also protects “life style –related” diseases such as cardiovascular disease and age related macular degeneration due to their antioxidant activity and pro vitamin A function [12]. They are used as colorants in the food industry to pigment salmon, trout and poultry flesh (or) to identify the colour of egg yolk [13].

The actual sale of carotenoids is estimated to be approximately US$500 million and the market is rising. Increasingly health-conscious consumers now actively seek functional foods and it is likely to trigger an increase in the demand for carotenoids in the food industry which is expected to lead to rapid sales growth of carotenoids [13]. The carotenoids market has been forecast to reach a value of $1.2 billion by 2018, driven by the growing demand from end-use applications such as food, animal feed and pharmaceuticals.

Recent developments in the molecular biology of carotenoids biosynthesis from organisms that accumulate different carotenoid product have provided a variety of genes [14] that can be employed as tools for a new strategy of heterologous expression in different host organisms. Engineering of microbial pathway enzyme can produce high amount of carotenoids in industrial process. The present study was aimed at isolation of bacterial isolates from different environment source, capable of producing carotenoids of possible commercial importance. Bacterial isolates were classified and identified through morphological and biochemical characteristics.

Materials and Methods

Soil sample collection and isolation of yellow pigmented bacteria

Soil samples were collected from various locations with different environmental condition of Salem district. Samples were collected by scraping of the soil surface with sterile spatula and about 10g of soil were obtained from a depth of 2-5cm. Bacteria present in the soil were isolated by serial dilution and spread plated on LB medium (g-1 peptone-10, NaCl- 10, yeast extract-5 and pH 6.8 ± 0.2) and incubated overnight at 36°C. Basic biochemical tests like gram’s staining, motility, Indole production, MR-VP, citrate utilization, catalase production, oxidase, Nitrate reduction, mannitol fermentation were performed based on Bergey’s manual and the results were observed.

Keywords: Yellow pigment; Carotenoid; DPPH

Abstract

The aim of the present was to isolate the microorganisms from different environment which is capable of producing high carotenoid pigments. Forty one soil samples were collected from different areas under different environmental and different climatic conditions. A total of 24 yellow pigmented colonies forming cocci shaped, gram positive bacteria were isolated. All the twenty four isolates were then subjected for carotenoid extraction using methanol as a solvent. High carotenoid producing isolates were confirmed by biochemical characterization and Mannitol salt growth. Of the 24 isolates tested, YCD3b showed highest production of carotenoid and it was confirmed through spectrophotometric analysis. Free Radical Scavenging Activity was studied by DPPH method. Isolate YCD3b displayed higher quantity of free radical scavenging activity compared to others isolates. Analysis of the peaks which were obtained at 450 nm indicated the presence of pigment, carotenoid.
Extraction of carotenoid form yellow pigmented bacterial isolates

The yellow pigmented bacterial isolates were grown in LB broth in a rotary shaker at 180rpm in 37°C for three days. After three days, cells were harvested by centrifugation at 8,000 rpm for 15 minutes. Then the pellet was washed with sterile distilled water and spin for 15 minutes at 4,000rpm. Five ml of methanol was added to the pellet and suspended it. Then it was incubated in a water bath at 60°C for 15 minutes until all visible pigments are extracted and centrifuged at 4,000 rpm for 15 minutes. The colored supernatant was separated then it was filtered through Whatman no. 1 filter paper [15]. The carotenoid extracts were analyzed by scanning the absorbance in the wavelength region of 450 nm using the spectrophotometer. The total carotenoid content in the methanol extract was estimated by measuring the absorbance at λmax (450 nm)

DPPH (1, 1-Diphenyl-2-picryl-hydrazil) free radical scavenging activity

The free radical scavenging activity of the fractions of methanol extract of carotenoid was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH), solution of DPPH (0.1 mM) in methanol was prepared; 1 ml of the solution was added to 3 ml of the fraction in methanol at different concentrations (25-500 mg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a UV-Visible Spectrophotometer [15]. The percent DPPH scavenging effect was calculated using the following equation:

\[
\text{DPPH scavenging effect} \% = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

Where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the standard sample or fraction. All the experiments were carried out in triplicates and the standard deviation was calculated. The significance of differences (P<0.05) between mean values obtained from the experiments was determined.

Results and Discussion

Soil samples were collected from different environmental conditions such as fertile land, wasteland, sewage areas and locations of Salem district viz, Yercaud hills, Periyar University campus and Kolli hills. A total of 41 soil samples were collected and subjected for isolation. Soil samples and locations are listed in Table 1. From the 41 soil samples, 24 yellow pigmented colonies were isolated. The microscopic observation shows that they are arranged in clusters. Bhonsale [16] also suggested that carotenoid production of the organism was mainly dependent on the environmental conditions and the composition of the culture medium and solvent. In our findings predominant numbers of the yellow pigmented colonies were obtained from the soil samples of fertile land and hill stations where the climate is slightly colder. The findings were also assisted as given by Bhonsale [16] where he emphasized on the effect of low temperature that induced an increase in carotenoid accumulation and he also added that it is mainly due to adaptation response that compensated the decreased functionality of biological membrane due to low temperature. The strains were further identified by colony morphology and biochemical identification. Preliminary morphological observations revealed that the colonies were circular, convex and yellow in color. Gram staining showed that the bacterium was gram positive, cocci. Results are given in the Table 2. Gargi et al. [17] reported that the yellow pigmented cocci shaped gram positive bacterium was obtained from soil in around Durgapur, West Bengal and they are maintained in Brain heart infusion agar.

Carotenoid extraction for the yellow pigmented isolates was carried out using methanol as a solvent. Out of 24 isolates only 8 isolates showed high amounts of carotenoid content viz., YCD3b>PCNS>NG P>YCT2>KH4>YCD3a>SK12a>YCD2a (Figure 1). From this results, isolate YCD3b produced significantly (P <0.05) higher amount of carotenoids when compared to other isolates (Figure 1). All pigmented bacterial isolates need not to be carotenogenic. With few exceptions carotenoids are lipophilic. They are insoluble in water and soluble in organic solvents [18]. The absorption spectrum of methanol extract of the cell pellet from the bacterial isolates showed maximum absorption at 450 nm which was identical to the absorption spectrum of β-carotene reference sample. The absorption maximum of methanol extract of YCD3b isolate is also very close to that of the standard β-carotene (Figure 2). Rodriguez-Amaya et al. [19] and Philipp [20] also suggested the absorption spectra of β-carotene to be around 450nm.

Indole, Methyl Red, Voges Proskauer tests showed negative for all

<table>
<thead>
<tr>
<th>Sampling area</th>
<th>Soil type/climatic (°C)</th>
<th>Geo-Location</th>
<th>Number of selected place</th>
<th>Number of strains collected</th>
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</thead>
<tbody>
<tr>
<td>Fertile land</td>
<td>Red soil/33</td>
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<td>11</td>
</tr>
<tr>
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<tr>
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<td>Red soil/35</td>
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<tr>
<td>Sewage</td>
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<td>11.742672 °N, 78.04723 °E</td>
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Table 1: Soil sample collection and isolation of yellow pigmented bacteria.

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Gram staining</th>
<th>Indole</th>
<th>MR</th>
<th>VP</th>
<th>Citrate utilization</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Nitrate reduction</th>
<th>Grown in mannitol salt agar</th>
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<tbody>
<tr>
<td>SK12a</td>
<td>Cocci,+</td>
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<td>-</td>
<td>-</td>
<td>+</td>
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<td>+</td>
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<tr>
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<td>-</td>
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<tr>
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</tbody>
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*, tested positive/used as substrate; -, tested negative/not utilized as substrate, MR- Methyl Red, VP- Voges-Proskauer

Table 2: Morphological and Biochemical characterization of yellow pigment producing isolates.
of the 8 isolates. But citrate and the catalase test showed positive. For the differentiation of Staphylococcus sp. from Micrococcus sp., oxidase, nitrate reduction test and mannitol salt agar growth were performed. All the 8 strains showed Oxidase negative and Nitrate positive results. But in Mannitol salt growth YCD2a did not grow. The growth of KH4 as white and YCT2, YCD2a, YCD3a, YCD3b, PCNS, NGP, SK12a as yellow in the medium were observed. From that KH4, YCD2a, YCT2, YCD3b, YCD3a, PCNS, NGP, SK12a strains were identified as Staphylococcus sp. according to Bergey’s manual. The results were given in the Table 2.

DPPH is the stable radical and is frequently used for evaluating the antioxidant activity of natural colorant products. Nishino [21] reported that DPPH radical is known to be stoichiometric ally decolorized by potent reducing substances and antioxidants such as cysteine, glutathione, ascorbic acid and tocopherols. Among the methanolic extracts of the eight isolates, the extract of YCD3b showed significantly higher amount (78%) of DPPH free radical scavenging activity when compared to other tested isolates (Figure 3). The methanol extract showed greater radical scavenging effect. Sasidharan [15] selected the highest carotenoid producing Exiguobacterium sp. for radical scavenging analysis which was isolated from air and soil samples. The strain has a total scavenging ability of around 70%. The DPPH free radical scavenging capacity of the methanol extracts showed in a concentration dependent manner [21]. Generally carotenoid is potential for antioxidants, many a times in in vivo, they lack such properties because of pro-oxidant effect [22]. However, carotenoid compounds have a significant role in anti-oxidant and anti-carcinogenic characteristics [23].

**Conclusion**

The results of our study show that the isolate (YCD3b) screened from Yercaud hills having mild climatic conditions produced a considerable amount of carotenoid. It can be used as a potential source for pharmaceutical and other cosmetic industries. In addition, pigment extracted from YCD3b showed better activity against free radicals. Recent developments in the molecular biology of carotenoid biosynthesis from organisms that accumulated different carotenoid product have provided a variety of genes that can be employed as tools for a new strategy of heterologous expression in different host organisms. Engineering of microbial pathway enzyme can produce high amount of carotenoids in industrial process from this YCD3b strain.

**Acknowledgment**

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**References**


