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# EXTRACTION AND CHROMATOGRAPHIC ANALYSIS OF β- CAROTENE PRODUCED BY RED YEASTS

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## ABSTRACT

Carotenoid diversity is important because of both its biotechnological potential and its role in understanding the evolution of secondary metabolism. The present study involves chromatographic profiling of the pigment extracts of the 14 wild type red yeast isolates in comparison with type culture *Rhodotorula glutinis* NCIM 3353. The HPTLC and HPLC analysis revealed presence of β -carotene in most of the yeast isolates. The chromatogram of the 14 wild type isolates along with the type strain *Rhodotorula glutinis* NCIM 3353 was scanned at 430 nm for separated bands of various carotenoids present in the pigment extracts. The approximate concentration of β-carotene in the type strain *Rhodotorula glutinis* NCIM 3353 was calculated to be 35.11μg $ml^{-1}$, amounting to 69% of the total carotenoids present in the sample. From the chromatograms higher concentration of β-carotene (219.5 μg $ml^{-1}$) was found in the pigment extract of soil isolate S1, *Rhodotorula mucilaginosa* MTCC 11823.

1. **KEYWORDS:** Carotenoids; chromatographic profile; β- carotene; *Rhodotorula mucilaginosa.*

1. **Abbreviations: HPTLC**- High Performance Thin Layer Chromatography.

## 2. Introduction

Carotenoids are the most pronounced, naturally occurring red or yellow pigments that occur widely in plants and animals. They are synthesized in plants and in some microorganisms and are only introduced with diet into humans and animals, which are incapable of their *de novo* synthesis.[1] Carotenoids are notable for their wide distribution, structural diversity and varied functions. They are a class of tetraterpenes with a characteristic isoprenoid polyene chain connecting the two end groups which may be cyclic or acyclic. More than 600 carotenoids have now been isolated and characterized from natural sources. This includes the enormous variety of carotenoids in algae, bacteria, yeast and fungi.[2,3] Due to the recent discovery of their anticancer and antioxidant properties, wider use of carotenoids as pharmaceuticals and nutraceuticals is expected. The synthesis of different carotenoids by several coloured yeast species has posed these microorganisms as a potential source of pigments.[4] Oxidative stress, favoring disease progression by a rapid degeneration of endothelial cell function is deeply involved in Systemic Sclerosis pathogenesis, Carotenoids may help in reverting endothelial dysfunction and damage, scavenging lipid peroxidation and reducing multiple episodes of hypoxia-reperfusion injury.[5] Studies suggest that high doses of beta-carotene may make people with erythropoietic protoporphyria, a rare genetic condition that causes painful sun sensitivity, as well as liver problems, less sensitive to the sun.[6] Higher dietary levels of antioxidants like carotenoids may reduce the risk of progression of age-related macular degeneration.[7] Carotenoids are known to lower the risks of prostrate cancer[8,9]; gastrointestinal cancers[10]; Estrogen negative breast cancers,[11] improve lung function[12]; cardiovascular disease and cancer mortality.[13]

Carotenoid diversity is important because of both its biotechnological potential and its role in understanding the evolution of secondary metabolism. Ascertaining the extent of carotenoid diversity from the literature is problematic due to the focus of most high-resolution studies on a single strain and/or the utilization of methods having insufficient resolution to differentiate between related compounds. Hence, in the present study the chromatographic profiling of the pigment extracts of all the wild type isolates in comparison with type culture *Rhodotorula glutinis* NCIM 3353 was performed.

Chromatography based analyses of crude samples for carotenoid composition is important in food chemistry and medicine. A simple HPTLC chromatographic method is sufficient because most yeasts tend to produce only a limited number of carotenoids; it is desirable

because it can be used even by non- specialized laboratories such as those engaged in taxonomic or phylogenetic problems, as a powerful tool to assess the pigment diversity of these organisms.[14]

## 3. MATERIALS AND METHODS

### 3.1. Chemicals

β-Carotene standard was obtained from Sigma- Aldrich. Acetonitrile, methanol, dichloromethane, hexane and ethyl acetate were of HPLC grade (Merck).

### 3.2 Extraction of cartotenoids

The 14 selected isolates of red pigmented yeasts were used in the present study (Table I). The method of extraction utilized here was a modified approach.[15] The yeast cells were harvested after five days of growth, by centrifugation (4500×g for 10 min) and washed three times with distilled water. Cell dry mass was determined after drying at 105 ºC. The yeast biomass was then disrupted by vortexing with glass beads (0.4 mm, 10 % w/v) in acetone for 15 minutes. Each suspension was separated by centrifugation and the pigment contained in the acetone was recovered and the supernatant was then partitioned with petroleum ether, to extract all the pigment.

### 3.3 Chromatographic Separation

β- carotene identification was performed by HPTLC following method by Perrier *et al.*[16] (1995). *Rhodotorula glutinis* NCIM 3353 strain carotenoid pigment profiles were used for matching. The carotenoid samples derived from various pigmented isolates were applied in 6 mm band width. The standard β-carotene sample was loaded in triplicates to derive approximate concentration of the same in the various pigment samples. Chromatographic runs were performed on 20x10 cm Silica gel G plates with mobile phase toluene: acetone (4:1). The separated pigment bands were best visualized at 430 nm and hence were scanned at 430 nm to obtain densitograms.

High-Performance Liquid Chromatography (HPLC), samples and the β-carotene standard (Sigma-Aldrich) were analyzed by diode-array UV detector (Waters 990). Carotenoids were separated by HPLC analysis on a reversed-phase Zorbax ODS C18 column (250×4.6 mm; 4.65µm particle size), Agilent, USA and sphere diameter 5µm. The temperature of the column was maintained at 20°C. The mobile phase was an isocratic solvent system consisting of acetone - water (67.4: 32.6, v/v) that was delivered by a LaChrom pump (L-7100) at a flow rate of 0.5 ml $min^{-1}$ and monitored at 430 nm, using $D_2$ lamp. HPLC device (LC Autosampler, LC Pump and UV/Vis detector (L-7420) was from Merck Hitachi LaChrom. To protect the column, a guard column of the same material was used. The samples were redissolved in 100 µl of mobile phase directly before the analysis and 20 µl was subjected to isocratic separation.

Conditions for the separation of carotenoids by HPTLC.

| Sr. No. | Parameter | Description |
|---|---|---|
| 1. | Stationary Phase | TLC Silica gel G60 $F_{254}$ MERCK |
| 2. | Mobile Phase | Toluene: acetone (4:1) |
| 3. | Sample applicator | CAMAG LINOMAT 5 |
| 4. | Development chamber | CAMAG Twin Trough Chamber (10X10 cm) |
| 5. | Band size | 6.0 mm |
| 6. | Space | 12 mm |
| 7. | Spotting volume | 10 µL |
| 8. | Spotting speed | 100 nL/sec |
| 9. | Development distance | 80 mm |
| 10. | Densitometric scanner | CAMAG TLC Scanner 3 |
| 11. | Chromatographic evaluation | Software (winCATS) |
| 12. | Lamp | Tungsten |
| 13. | Slit dimension | 10 mm |
| 14. | Scanning wavelength | 430 nm |

## 4. RESULTS AND DISCUSSION

The chromatogram of the 14 wild type isolates along with the type strain *Rhodotorula glutinis* NCIM 3353 was scanned at 430 nm for the observation of separated bands of various carotenoids present in the pigment extracts. The retardation factor of one of the separated bands from all the yeast isolates pigment extract was found to be comparable with the standard β-carotene, indicating presence of β-carotene in their extracts. The standard β-carotene (500µg$ml^{-1}$) was found to have the $R_f$ value 0.83 (Plate1). The approximate concentration of β-carotene in the type strain *Rhodotorula glutinis* NCIM 3353 and selected yeast isolates pigment extracts was calculated (Table I). All the samples, except *Rhodosporidium paludigenum,* showed presence of two distinct bands in the densitograms.

Diversity of carotenoids is important because of both its biotechnological potential and its role in understanding the evolution of secondary metabolism. The extent of carotenoid diversity is difficult to be ascertained from the literature, due to the fact that most high-resolution studies focus on a single strain and the utilization of methods having insufficient resolution to differentiate

between the related compounds. Therefore in the present study HPTLC methods were used to study the yeast carotenoid pigments. The HPTLC methods used for determining the carotenoid profiles of various yeast isolates are simple, rapid, selective, sensitive, economical, reliable and reproducible.

The $R_f$ value of the β-carotene fraction of the pigment extracts was found to be 0.83± 0.04, when the toluene: acetone (4:1) mobile was used for HPTLC. According to Rodriguez-Amaya (2001)[17] the carotenoids tend to run along with the solvent front. Perrier *et al.* (1995)[16] and Latha *et al.* (2010)[18] showed the $R_f$ value of the β-carotene to be 0.92 in petroleum ether: acetone (80:20). Sperstad *et al.* (2006)[19] used hexane: acetone for yeast pigment separation to get β-carotene band $R_f$ value 0.90. Park *et al.* (2007)[20] reported that the chromatographic analysis of the crude extract from *R. glutinis* showed similar three carotenoid pigments, viz. β-carotene, torulene and torularhodin.

The HPLC elution time of the β-carotene standard was found to be 3.01 min. The HPLC of the pigment extracts of various yeast isolates showed β-carotene peaks at the elution time values ranging 3±0.3 mins (Table II). The pigment extract of all the yeast isolates showed presence of a single peak in their chromatogram with the retention time of 3.01±0.17, which is comparable with that of standard β-carotene. These results are in agreement with earlier work that analyzed yeast strains isolated from different environments worldwide, suggesting a general qualitative constancy in the composition of carotenoid pigments for the genera studied. However, the HPLC analysis revealed primarily the β-carotene peak in most of the pigment extracts of various isolates.

**Table I: HPTLC analysis of carotenoids extracted from pigmented yeast isolates and *Rhodotorula glutinis* NCIM 3353 along with standard β-carotene.**

| Sample | Rf | AUC | Area % | Approximate β-carotene concentration (μg $ml^{-1}$) |
|---|---|---|---|---|
| Std. β-carotene | 0.83 | 5774.0 | 100 | 500 |
| *Rhotorula glutinis* NCIM 3353 | 0.52 | 961.6 | 31.45 | |
| | 0.82 | 2096.0 | 68.55 | 35.11 |
| *Rhodotorula mucilaginosa* | 0.40 | 417.5 | 13.73 | |
| | 0.84 | 2622.3 | 86.27 | 219.5 |
| *Rhodotorula minuta* | 0.49 | 782.3 | 21.36 | |
| | 0.82 | 2880.6 | 78.64 | 48.24 |
| *Rhodotorula glutinis* | 0.44 | 2808.7 | 50.70 | |
| | 0.80 | 2731.6 | 49.30 | 91.48 |
| *Rhodotorula aurantiaca* | 0.40 | 618.5 | 12.29 | |
| | 0.81 | 4414.8 | 87.17 | 184.80 |
| *Sporobolomyces roseus* | 0.43 | 1702.3 | 64.59 | |
| | 0.83 | 933.3 | 35.41 | 15.62 |
| *Sporidiobolomyces johnsonii* | 0.48 | 2389.0 | 53.74 | |
| | 0.81 | 2056.8 | 46.26 | 34.44 |
| *Rhodotorula minuta* | 0.39 | 588.7 | 10.85 | |
| | 0.82 | 4837.9 | 89.15 | 202.48 |
| *Sporobolomyces salicinus* | 0.43 | 2423.4 | 40.95 | |
| | 0.81 | 3494.1 | 59.05 | 58.50 |
| *Sporobolomyces salicinus* | 0.14 | 1356.1 | 19.26 | |
| | 0.48 | 3844.7 | 54.62 | |
| | 0.80 | 1838.6 | 26.12 | 30.79 |
| *Sporidiobolomyces salmonicolor* | 0.41 | 504.6 | 6.84 | |
| | 0.82 | 6871.8 | 93.16 | 115.06 |
| *Sporidiobolomyces ruineniae* | 0.58 | 399.6 | 44.71 | |
| | 0.85 | 494.1 | 55.29 | 41.36 |
| *Rhodotorula acheniorum* | 0.62 | 693.4 | 35.20 | |
| | 0.86 | 1276.8 | 64.80 | 21.37 |
| *Rhodosporidium paludigenum* | 0.83 | 747.0 | 100 | 31.27 |
| *Sporobolomyces novazealandicus* | 0.40 | 1397.3 | 56.36 | |
| | 0.83 | 1081.9 | 43.64 | 18.12 |

**Table II: HPLC analysis of carotenoids extracted from pigmented yeast isolates and *Rhodotorula glutinis* NCIM 3353 along with standard β-carotene.**

| Sample | $R_t$ | AUC | Approximate β-carotene concentration(μg $ml^{-1}$) |
|---|---|---|---|
| Standard(100 μg $ml^{-1}$) | 3.01 | 4706 | 100.00 |
| *Rhotorula glutinis* NCIM 3353 | 2.87 | 60649 | 32.22 |
| | 4.61 | 20725 | - |
| *Rhodotorula mucilaginosa* | 3.01 | 227622 | 241.84 |
| *Rhodotorula minuta* | 3.03 | 63641 | 33.81 |
| *Rhodotorula glutinis* | 3.08 | 20920 | 111.13 |
| *Rhodotorula aurantiaca* | 3.03 | 28875 | 153.39 |
| *Sporobolomyces roseus* | 2.90 | 25023 | 13.29 |
| *Sporidiobolomyces johnsonii* | 3.02 | 23430 | 37.34 |
| *Rhodotorula minuta* | 3.10 | 25349 | 179.55 |
| *Sporobolomyces salicinus* | 3.17 | 38636 | 54.73 |
| *Sporidiobolomyces salmonicolor* | 3.02 | 18846 | 26.71 |
| *Sporidiobolomyces ruineniae* | 3.05 | 15016 | 106.36 |
| *Rhodotorula acheniorum* | 3.01 | 19447 | 20.66 |
| *Rhodosporidium paludigenum* | 3.04 | 49096 | 44.73 |
| *Sporobolomyces novazealandicus* | 3.02 | 48550 | 25.79 |

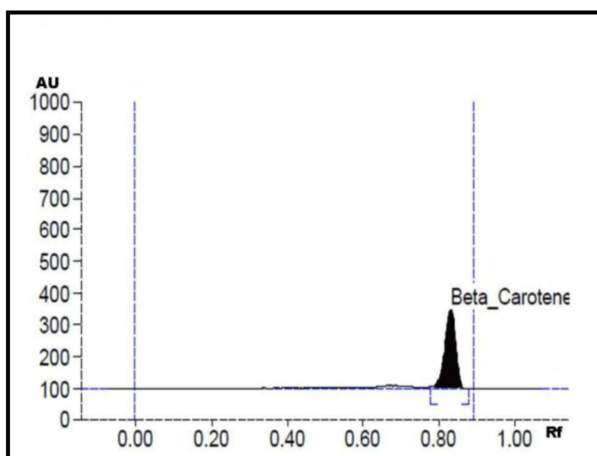


**Plate 1: HPTLC densitogram of standard β-carotene.**

## 5. CONCLUSION

The method of pigment extraction and HPTLC analysis showed presence of various types of carotenoids in the red yeast isolates under study. The HPLC analysis of the pigment extracts from various red yeast isolates selected for the study showed presence of β-carotene in all. Most of the isolates showed presence of another minor pigment in addition to β-carotene. Out of the 14 yeast isolates, *Rhodotorula mucilaginosa* synthesized highest amount of total carotenoids and also contained highest amount of β-carotene and hence the yeast can be a potential source of this therapeutic biocompound.

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## 7. CONFLICT OF INTEREST

There is no conflict of interest with any funding source for the study undertaken.

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