Isolation and identification of carotenoid-producing *Rhodotorula* sp. from soils collected in the coastal environment of Kien Giang and Tra Vinh provinces

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Abstract:

***Rhodotorula* is a genus of marine yeasts that is considered the most suitable for carotenoid production. In this study, collected soils at Kien Giang and Tra Vinh provinces were cultured on yeast extract peptone dextrose (YEPD) agar for 72 hours at room temperature. Twenty-three isolates exhibiting yellow, orange, and pink pigments were tested for physiological and biochemical characteristics such as carbohydrate fermentation and assimilation, nitrate assimilation, urease production, and growth temperature. Their oval dimensions were 4.24x3.64 µm for isolate 5S1-T and 3.88x3.17 µm for isolate 5S4-T. The two isolates could assimilate carbohydrate and nitrate sources and produce urease. The ITS rDNA sequence of isolates 5S1-T and 5S4-T both had 100% matches to *Rhodotorula sphaerocarpa* CBS:5940 and *Rhodotorula sphaerocarpa* CBS:5939, respectively. The maximum absorbance wavelengths of the solution extractions from the 5S1-T and 5S4-T were 496 and 487 nm, respectively. The total carotenoid content of strain 5S1-T was found to be the highest (1.81 mg/l) followed by strain 5S4-T (1.16 mg/ml). The antioxidant abilities of the 5S1-T and 5S4-T were demonstrated by scavenging the free radical DPPH and ferric reducing capacity. The free radical scavenging activity of the extract from isolate 5S1-T (96.15%) was higher than that of isolate 5S4-T (88.61%) at 0.14 mg/ml. The absorbance at 700 nm of the extracts of both 5S1-T and 5S4-T significantly increased from 0.05 to 0.3 mg/ml. The two strains 5S1-T and 5S4-T of *Rhodotorula sphaerocarpa* discovered in this study could contribute to carotenoid and antioxidant-producing yeasts for industries in the future.**

Keywords: **antioxidant, carotenoid, *Rhodotorula*, yeast.**

Classification numbers: **3.5, 5.3**

1. Introduction

Rhodotorula sp., in the phylum *Basidiomycota* and family *Cryptococcaceae*, is a potential genus for carotenoid production and cultivation on an industrial scale. Carotenoids are natural bioactive compounds that are capable of antioxidant, antibacterial, and antifungal activities. Because of their biological role as vitamin A precursors in humans and their antioxidant properties preventing some forms of cancer, carotenoids have been indicated as a one of the most valuable compounds for applications in food, feed, pharmaceutical, and chemical industries. Carotenoids are also useful as a natural colorant and can be used to avoid artificial colorants in food and beverage industries [1-4].

The marine environment has proven to be a rich source of both bacteria and yeast diversity with bioactive secondary metabolite substances. Marine organisms have recently been concerned with many useful characterizations that can be modified for specific uses. Over 300,000 described species have been discovered in the ocean, which makes up more than 70% of the earth's surface including bacteria and yeast [5].

Rhodotorula sp., which was isolated from soils of the marine zone, has been historically reported in many countries [6-8]. It is commonly known that *Rhodotorula* sp. is being evaluated as a potential natural resource for industrial applications such as the production of carotenoids, bioethanol, enzymes, protein, and silver nanoparticles [8-12]. However, in Vietnam, there is a focus on investigations of potential features of *Rhodotorula* originating from oceanic areas as well as mangrove sediments. As for marine yeasts, *Rhodotorula* sp. will be a novel resource for carotenoid production.

Therefore, this research was conducted to isolate and identify carotenoid-producing *Rhodotorula* sp. from the coastal environments of the Kien Giang and Tra Vinh provinces. The aim is to isolate and characterise novel

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strains that can produce carotenoids as a group of natural bioactive compounds with potential antioxidant activities and applications for industrial production.

2. Materials and methods

2.1. Sample collection

Soil samples were collected about 50 cm from the face of the waters in the coastal environments of the Kien Giang and Tra Vinh provinces. The soils were kept in a cool box and transported to the laboratory where they were stored at 4°C until further use. This is a suitable temperature to conserve *Rhodotorula* yeast in the samples [13].

2.2. Methods

Isolation of *Rhodotorula:* About 10 g of soil was transferred to 90 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotator shaker (150 rpm) for 30 min at room temperature. The supernatant in the soil was diluted up to 10^{-3} dilutions. Then, 100 µl of each dilution was spread on a YEPD agar plate supplemented with streptomycin sulphate (250 mg/l) and penicillin (250 mg/l). All plates were incubated at room temperature for 7 days. Observing the morphology as well as colours of colonies every 24 hours was conducted to isolate the colonies that contain the pink, red, yellow, and orange colour and properties of yeast including fermentation of carbohydrates (glucose, sucrose, and mannitol), assimilation of carbohydrates and nitrate, urease production, and growth at different temperatures [13-16].

The isolates from *Rhodotorula* sp. were determined and selected for analysis based on N.J.W. Kreger-Van Rij's (1984) [17] description of its micromorphological, physiological, and biochemical characteristics.

2.3. Taxonomy

DNA extraction: Yeast genomic DNA extraction was conducted according to Y.J. Zhang, et al. (2010) [18] with the following modifications. After 3 days of cultivation, 7-10 yeast colonies on solid YEPD medium were placed in a 2-ml eppendorf tube. In the next step, 1 ml of 1X phosphate-buffered saline was added to the yeast and then centrifuged at 10,000 rpm for 4 min (eppendorf centrifuge 5810R, Germany). Afterwards, the supernatant was discarded, the yeast precipitate was retained, and 0.5 ml of TE buffer (50 mM Tris-HCl pH 7.4, 20 mM EDTA) was added followed by the addition of phenol, chloroform, and isoamyl alcohol in a ratio of 25:24:1, respectively, with an equivalent volume to the mixture, which was then placed on ice and centrifuged at 12,000 rpm for 5 min. Moreover, the mixture was incubated at 65°C for 30 min. Furthermore, the mixture was placed in an ice bath to retain heat and centrifuged at 12,000 rpm for 5 min. As a result, the supernatant was transferred to another clean tube, mixed with the corresponding volume of absolute ethanol, and incubated overnight at -20°C. The mixture was centrifuged at 12,000 rpm for 5 min. The supernatant was removed, then 500 µl of 70% ethanol was added to the mixture to wash the pellet (repeated 3 times). Finally, the pellet was air-dried, dissolved in 100 µl 0.1 X TE, and stored at -20°C.

Amplification of ITS gene by PCR: Microscopic and macroscopic features of the isolate were examined by classical microbiological techniques. Then, the isolates were identified by using the molecular biological method [13]. The following universal primers ITS 1 and ITS 4 were used for the amplification of ITS region of the isolates:

Forward primer ITS 1: 5'-TCCGTAGGTGAACCTGCGG-3'

Reverse primer ITS 4: 5'-TCCTCCGCTTATTGATATGC-3'.

The PCR consisted of an initial denaturing step of 5 min at 94°C followed by 35 cycles (50 s at 94°C, 50 s at 54°C, and 50 s at 72°C) finished by a final extension step at 72°C for 10 min. A GeneAmp® PCR system ABI 9700, USA was used for the amplification of the ITS region of the samples. After that, the PCR products were detected by electrophoresis (Gel tank MGU-502T, CBS Scientific, and basic power supply, Biorad, USA) through 1.5% agarose gels in Tris-borate EDTA solution and were visualized by staining with SyBr Green. The products were then conducted for sequencing by using Sanger Technique (ABI 3500, Applied Biosystem, USA) at Nam Khoa Biotech Company. The ITS sequence was searched for the closest homology sequence using the BLAST on the GenBank (NCBI).

Extraction and quantification of total carotenoids: The isolates were cultured on YEPD agar a pH 5.6 and incubated for 3 days on the shaker (Thermostable Shaker KS4000, IKA, Germany) (180 rpm/min) at room temperature. After that, the entire broth was centrifuged at 8,000 rpm for 10 min at 25°C. The cell pellets were collected and extracted with 1 ml of dimethyl sulfoxide (DMSO) preheated to 55°C (Waterbath Memmert One, Germany). After centrifugation, the supernatant pigment was pipetted off and the extraction in DMSO was repeated three times. The maximum absorbance wavelength and the total carotenoid content were determined by measuring the absorbance value using a spectrophotometer according to H.M. Kanzy, et al. (2015) [19]. The total carotenoid content was calculated according to the following equation:

$$C = \frac{A \times V \times 10^6}{E_{1cm}^{1\%} \times 100}$$

where C is the total carotenoid concentration (mg/l); A is the maximum absorbance value; V is the total volume (ml); $E_{1cm}^{1\%}$ is the average absorption coefficient of carotenoids in DMSO (2,040).

2.4. Antioxidant assay

Free radical scavenging capacity DPPH assay: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was carried out based on the loss of DPPH colour when a solution of DPPH is in contact with a substance that is capable of antioxidant activity. The evaluation of the antioxidant activity assay was measured according to M.P. De Torre, et al. (2019) [20] with some modifications. A 200-µl sample was added to 200 µl DPPH (0.05 mg/ml) in absolute ethanol. Then, the mixture was incubated for 60 min at room temperature. The extract solution was prepared in DMSO. Stock extracts with various concentrations were made. Finally, the mixture was diluted with 600 µl distilled water and the absorbance was measured at 517 nm. The percentage of DPPH scavenging free radicals was calculated by the following equation:

$$I\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where I is the percentage of inhibition (scavenging free radicals DPPH); $A_{control}$ is absorbance (ABS) of control at 517 nm; A_{sample} is the ABS of the reaction mixture in the presence of extract after the reaction was stopped at 517 nm.

Ferric reducing antioxidant power (FRAP) assay: FRAP analysis was introduced to measure total antioxidant activity and is based on the ability of samples to reduce ferric ion Fe^{3+} to ferrous ion Fe^{2+}, forming a blue complex. The method was conducted according to a modified version of M. Vijayalakshmi and K. Ruckmani (2016) [21] method. The reaction includes 0.5 ml of various concentrations of samples and 0.5 ml of 1% $K_3Fe(CN)_6$ in 0.5 ml of 0.2 M sodium phosphate buffer (pH 6.6). The reaction mixture was vortexed and then incubated at 50°C for 20 min. At the end of incubation, 0.5 ml of 10% trichloroacetic acid (CCl_3COOH) was added to the mixture and centrifuged at 3,000 rpm for 10 min. A 0.5-ml volume of supernatant of was mixed with 0.5 ml deionised water and 0.1 ml 0.1% $FeCl_3$. The mixture was vortexed well by using a vortex shaker (Mixer Vortex MS2, IKA, Germany). The coloured solution was measured at 700 nm.

2.5. Statistical analysis

Mean values of the data were calculated using Microsoft Excel 2011. Statistical analysis was performed with SPSS version 20. Statistical analyses of the experimental data were handled utilising a one-way analysis of variance (ANOVA). The difference of means of the independent group was tested for significance by using Duncan's multiple range test and the least significant difference test (LSD).

3. Results and discussion

3.1. Isolation of Rhodotorula

The soil samples were collected from coastal areas in the Kien Giang and Tra Vinh provinces. There were 23 pigmented samples isolated from soil samples from Kien Giang and Tra Vinh, which are indicated in Table 1. To be more specific, 19 samples were isolated from soil samples from Kien Giang (82.61%) whereas 4 samples were isolated from soil samples from Tra Vinh (17.39%).

Table 1. Colony morphological characteristics of isolates and the location of the collected soil samples.

No.	Isolates	Province of collected soils	Colony colour	Colony shape	Colony margin	Colony elevation	Colony diameter (mm)
1	2S34-K	Kien Giang	Orange	Irregular	Undulate	Crateriform	0.40
2	2S341-K	Kien Giang	Orange	Irregular	Undulate	Crateriform	0.76
3	2S342-K	Kien Giang	Yellow	Irregular	Undulate	Crateriform	0.83
4	2S31-K	Kien Giang	Bold orange	Circular	Entire	Raised	0.38
5	2S36-K	Kien Giang	Orange	Irregular	Undulate	Raised	0.40
6	2S37-K	Kien Giang	Orange	Circular	Entire	Raised	0.72
7	4S6-T	Tra Vinh	Pink	Circular	Entire	Raised	0.30
8	4S7-T	Tra Vinh	Yellow	Circular	Entire	Umbonate	0.40
9	5S1-T	Tra Vinh	Bold orange	Circular	Entire	Raised	0.83
10	5S4-T	Tra Vinh	Bold orange	Circular	Entire	Raised	0.70
11	23S6-K	Kien Giang	Orange	Irregular	Undulate	Crateriform	0.50
12	23S61-K	Kien Giang	Orange	Circular	Entire	Umbonate	0.50
13	23S63-K	Kien Giang	Orange	Irregular	Undulate	Crateriform	0.30
14	23S69-K	Kien Giang	Orange	Circular	Entire	Raised	0.60
15	24S66-K	Kien Giang	Orange	Circular	Entire	Raised	0.45
16	24S67-K	Kien Giang	Orange	Circular	Entire	Umbonate	0.50
17	39S6-K	Kien Giang	Orange	Circular	Entire	Umbonate	0.73
18	39S61-K	Kien Giang	Orange	Circular	Entire	Raised	0.62
19	39S62-K	Kien Giang	Orange	Circular	Entire	Raised	0.53
20	39S64-K	Kien Giang	Orange	Circular	Entire	Umbonate	0.90
21	39S65-K	Kien Giang	Pale orange	Irregular	Undulate	Crateriform	0.60
22	39S66-K	Kien Giang	Orange	Circular	Entire	Umbonate	0.70
23	39S69-K	Kien Giang	Orange	Circular	Entire	Raised	1.50

Notes: S is soil; K is Kien Giang province; T is Tra Vinh province.

In Table 1, the colonies of 20 isolated samples had colours from yellow to bold orange. Their colony diameters ranged from 0.3 to 1.5 mm. There were three isolates with bold orange colour (13.04%), sixteen isolates with orange colour (69.57%), two samples with yellow colour (8.69%), one sample with pink colour (4.35%), and one sample with pale orange colour (4.35%). Additionally, 16 colony isolates had circular morphology (69.57%), whereas 7 colony isolates had irregular morphology (30.43%). In terms of colony elevation, there were 6 pigmented isolates with crateriform height, which accounted for 30%. Besides this, pigmented isolates with raised height had 11 isolates, which accounted for 47.83%.

After being cultured in YEPD agar medium for 3 days, 23 isolates that had orange, pink and yellow colours were separated and tested for morphological, physiological, and biological characterisation belonging to the yeast.

To identify the preliminary properties belonging to the yeast, the 23 isolates were observed under microscopy to evaluate the cell morphology of the pigmented isolates. As a result, eight pigmented isolates were morphologically similar to yeast, including isolates 2S31-K, 2S36-K, 2S37-K, 5S1-T, 5S4-T, 23S6-K, 24S67-K, and 39S65-K. Based on the results of the physiological and biochemical tests described in Table 2, the two isolates (5S1-T and 5S4-T) had some properties similar to those of the yeast genus *Rhodotorula*. To explain in more detail, 5S1-T and 5S4-T could assimilate carbohydrate sources, namely glucose, sucrose, and mannitol. But the isolates were not capable of fermentation of glucose nor sucrose, which is important to indicate in the classification of the genus *Rhodotorula*. Also, the assimilation of nitrate sources of isolates 5S1-T and 5S4-T was positive. The ability of urease production was recorded by colour changing when isolates 5S1-T and 5S4-T were cultured in a mineral medium containing urea. Regarding physiological characteristics, eight isolates were tested for growth at four temperatures, namely, 20, 25, 30, and 37°C. All isolates experienced good growth at 25 and 30°C. Moreover, the six isolates 2S31-K, 2S36-K, 2S37-K, 23S6-K, 24S67-K, and 39S65-K grew well at 37°C, except for isolates 5S1-T and 5S4-T. According to the taxonomy key in a study, almost all strains of the genus *Rhodotorula* either slowly grow or have no development at 37°C. This result is similar to the growth characterizations of *Rhodotorula mucilaginosa* CRUB 0138 and *Rhodotorula mucilaginosa* PYCC 5166 [1]. Only the two isolates 5S1-T and 5S4-T could grow at 20°C. This is clear evidence that isolates 5S1-T and 5S4-T fall into the group yeast. Nevertheless, isolate 24S67-K was able to produce urease to hydrolyse urea sources and assimilate glucose, sucrose, maltose, and nitrate sources. However good growth of the isolate was observed at 37°C. Therefore, it is unclear which characteristic to use to identify the isolate belonging to the yeast *Rhodotorula*. Therefore, further experiments are needed to accurately identify this isolate.

The isolates 5S1-T and 5S4-T developed a bold orange-coloured colony on YEPD agar medium after 48 hours and grew well after that. The colour of the colony became darker after 5 days. The colony had a circular shape with a mucous and smooth surface (Fig. 1).

Table 2. Physiological and biochemical characteristics of the yeast isolates.

No.	Characteristics \ Isolates	2S31-K	2S36-K	2S37-K	5S1-T	5S4-T	23S6-K	24S67-K	39S65-K
1	Glucose assimilation	+	+	+	+	+	+	+	+
2	Glucose fermentation	-	-	-	-	-	-	-	-
3	Sucrose assimilation	+	+	+	+	+	+	+	+
4	Sucrose fermentation	-	-	-	-	-	-	-	-
5	Mannitol assimilation	+	+	+	+	+	+	+	+
6	Mannitol fermentation	-	-	-	-	-	-	-	-
7	Nitrate assimilation	-	+	-	+	+	-	-	-
8	Urease production	-	+	+	+	+	+	+	-
9	Growth at 20°C	-	-	-	+	+	-	-	-
10	Growth at 25°C	+	+	+	+	+	+	+	+
11	Growth at 30°C	+	+	+	+	+	+	+	+
12	Growth at 37°C	+	+	+	-	-	+	+	+

Notes: +: positive; -: negative.

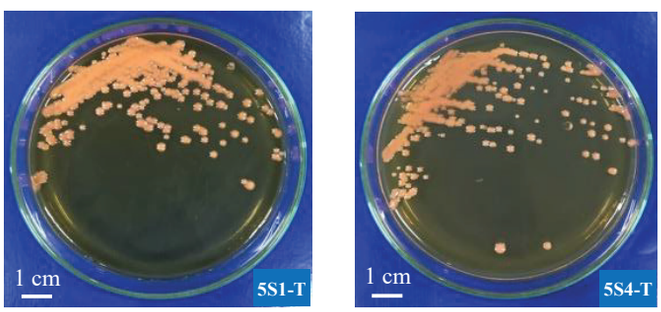

Fig. 1. Colony of isolates 5S1-T and 5S4-T on YEPD agar medium after 5 days.

Scanning electron microscopy (SEM) was applied to capture the cell morphology of yeast isolates. Both the two isolates had an oval shape; moreover, the cell diameter of the isolates were 4.24x3.64 (μm) for isolate 5S1-T and 3.88x3.17 (μm) for isolate 5S4-T, which is indicated in Fig. 2.

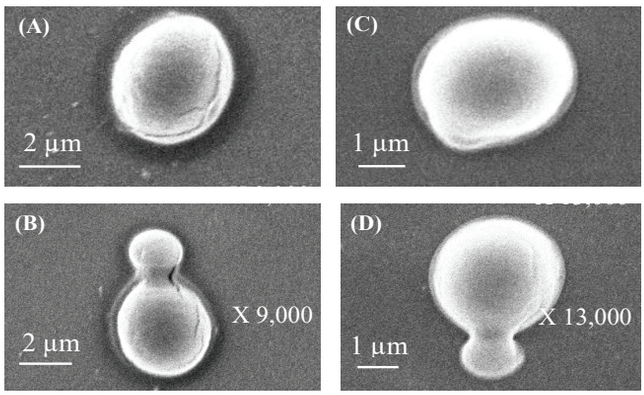

Fig. 2. Cells of isolates 5S1-T and 5S4-T under a scanning electron microscope. (A) The cell of isolate 5S1-T; **(B)** budding cell of isolate 5S1-T; **(C)** cell of isolate 5S4-T; **(D)** budding cell of isolate 5S4-T.

Overall, isolates 5S1-T and 5S4-T could assimilate glucose, sucrose, mannitol, and nitrate sources and hydrolyse urea, but could not ferment carbohydrate sources. Apart from that, their cell morphology was oval, and their type of reproduction was budding, which is a typical feature of yeast. Based on morphological, physiological, and biochemical characteristics, the two isolates 5S1-T and 5S4-T had some properties that were similar to those of the yeast genus *Rhodotorula*. However, the others showed distinct characteristics, so it is crucial to conduct further experiments to identify these.

Molecular biology Identification: Fig. 3 shows that the length of PCR products of the isolates 5S1-T and 5S4-T was approximately 750 bp through amplification of the ITS sequence to identify them accurately.

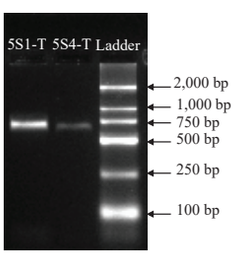

Fig. 3. PCR product of an ITS fragment of isolates 5S1-T and 5S4-T on agarose gel by analysing electrophoresis.

The sequence was compared with ITS sequences deposited in GenBank (NCBI) by using the BLAST tool. The ITS sequence of 5S1-T showed a 100% similarity to *Rhodotorula sphaerocarpa* 5940. The ITS sequence of 5S4-T showed a 100% similarity to *Rhodotorula sphaerocarpa* 5939 (Table 3). Although the two samples, 5S1-T and 5S4-T, also belong to the species *Rhodotorula sphaerocarpa*, the identification of the isolates was different based on the capacity of carotenoid production and growth rate of the colony. Therefore, they could be subspecies of *Rhodotorula sphaerocarpa*.

Table 3. ITS Sequence comparison of isolates on NCBI GenBank.

Isolates	Length	Species	Query cover	Percentage of identification	Accession
5S1-T	610 bp	*Rhodotorula sphaerocarpa* culture CBS:5940. Length: 622 bp.	100%	100%	KY104909.1
5S4-T	559 bp	*Rhodotorula sphaerocarpa* culture CBS:5939. Length: 577 bp.	100%	100%	KY104905.1

3.2. Determination of total carotenoid content

Table 4 provides the pigment extract of *Rhodotorula* sp. 5S1-T, which had a bold orange colour and maximum absorbance wavelength at 496 nm, which was the highest one. The total carotenoid content extracted from isolate 5S1-T was 1.81±0.003 mg/l, corresponding to a maximum absorbance of 0.31±0.001 mg/l, which was a significant difference between the isolates. The predicted carotenoid composition of extract 5S1-T was -carotene, -carotene, lutein, and lycopene. Besides, the 5S4-T extract of *Rhodotorula* sp. had a maximum absorbance wavelength at 487 nm followed by a maximum absorbance of 0.20±0.001, corresponding to a total carotenoid content of 1.16±0.007 mg/l. Based on the maximum absorbance wavelength of the extract, the carotenoid composition of isolate 5S4-T was predicted to be β-carotene and lycopene. These results are similar to studies from several other countries. In Argentina, it was reported by D. Libkind, et al. (2004) [1] that the content of total carotenoid extracted from *Rhodorotula mucilaginosa* CRUB 0138 was 1.04 mg/l. According to the 2016 research by P. Hanachi and F.S. Naghavi (2016) [22] in Iran, the total carotenoid contents of *Rhodorotula slooffiae* and *Rhodorotula mucilaginosa* were 1.216 and 0.604 mg/l, respectively. In another study, the carotenoid produced by *Rhodorotula mucilaginosa* was 8.11 mg/l at 96 hours of cultivation [23].

Table 4. Determination of maximum absorbance wavelength of extracts.

Extract of samples	Extract colour	Maximum absorbance wavelength of extract (nm)	Maximum absorbance (Abs)	Total carotenoid content (mg/l)	Possible carotenoid compounds	Reference
5S1-T	Bold orange	496	0.31±0.001[a]	1.81±0.003[a]	y-carotene, β-carotene, lutein, lycopene	[24] [25]
5S4-T	Bold orange	487	0.20±0.001[b]	1.16±0.007[b]	β-carotene, lycopene	
F			*	*		
CV (%)			0.57	0.57		

Notes: Numbers in a column with distinguished letters are significantly different at p<0.05 by using the LSD test; data were shown by MEAN±SE.

3.3. Antioxidant assay of extract

In this study, two samples of *Rhodotorula* isolated from Tra Vinh province were examined for their antioxidant potential under *in vitro* conditions. All of the samples were able to scavenge the radical DPPH and reduce ferric ions to ferrous ions. The results of antioxidant activity based on DPPH scavenging are given in Table 5. Generally, the inhibition of DPPH of the two extracts shows an upward trend, rising noticeably from 2.86±0.84% at 0.02 mg/ml to 96.15±0.31% at 0.14 mg/ml of extract of strain 5S1-T and from 2.19±0.43% at 0.02 mg/ml to 88.61±0.45% at 0.14 mg/ml of extract of strain 5S4-T. The percentage of observed concentrations of *Rhodotorula* sp. 5S1-T and *Rhodotorula* sp. 5S4-T extracts were a significant difference in statistics ($p<0.05$).

Table 5. Results of antioxidant activity of isolated extracts by using the DPPH.

Isolates / Concentrations (mg/ml)	Percentage of inhibition (%)	
	5S1-T	*5S4-T*
0.00	0.00±0.00^{h}	0.00±0.00^{h}
0.02	2.86±0.84^{g}	2.19±0.43^{g}
0.04	14.32±0.42^{f}	10.07±0.37^{f}
0.06	29.04±0.70^{e}	19.83±1.52^{e}
0.08	38.12±0.39^{d}	34.01±0.66^{d}
0.10	50.63±0.19^{c}	49.77±0.40^{c}
0.12	72.78±0.28^{b}	70.56±0.38^{b}
0.14	96.15±0.31^{a}	88.61±0.45^{a}
F	*	*
CV (%)	2.12	3.22

Notes: Numbers in a column with distinguished letters are significantly different at $p<0.05$ by using Duncan's multiple range test; data were shown by MEAN±SE.

To compare the antioxidant activity of extracts and positive controls, namely, vitamin C and commercial beta-carotene, the value of inhibition concentration at 50% (IC_{50}) was used, which is defined as the concentration of extracts necessary to scavenge the DPPH free radicals by 50%. Given in Fig. 4, the IC_{50} of beta-carotene was the strongest (0.002 mg/ml), followed by vitamin C (0.021 mg/ml), extract of *Rhodotorula sphaerocarpa* 5S1-T (0.093 mg/ml), and extract of *Rhodotorula sphaerocarpa* 5S4-T (0.096 mg/ml). Gerelmaa et al. reported that carotenoids extracted from *Rhodotorula glutinis* R12 could scavenge 50% of DPPH at 0.536 mg/ml [26]. A study by F.S. Naghavi (2015) [4] revealed that both strains, *Rhodorotula slooffiae* and *Rhodorotula mucilaginosa*, showed a significant antioxidant effect and had scavenging of 50% DPPH at 0.658 and 0.789 mg/ml, respectively. Using the same method, T.T. Men, et al. (2020) [27] reported that the ethanolic extract of *Kaempferia galanga* showed a scavenging ability of 50% DPPH at 2.404 mg/ml. It is indicated that carotenoids extracted from *Rhodotorula sphaerocarpa* 5S1-T and 5S4-T had high antioxidant activity.

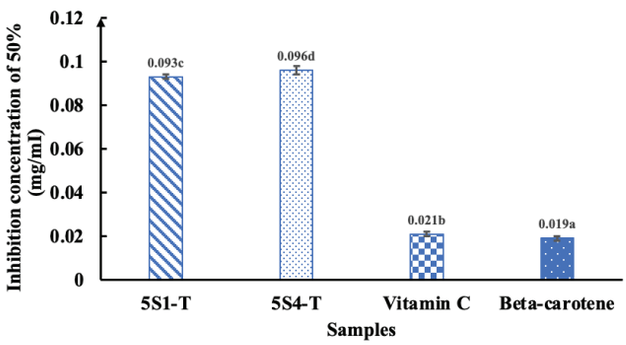

Fig. 4. The inhibition concentration of radical DPPH of 50% of samples (extract 5S1-T, extract 5S4-T, vitamin C, and beta-carotene).

To evaluate the antioxidant activity by using a reducing ferric ion assay, the absorbances of reducing the capacity of the extracts 5S1-T and 5S4-T were recorded and are presented in Table 6. The increased absorbance of the reaction mixture indicates raised reducing capacity [28].

Table 6. Results of antioxidant activity of isolated extracts by using the ferric reducing antioxidant capacity assay.

Isolates / Concentrations (mg/ml)	Absorbance at 700 nm	
	5S1-T	*5S4-T*
0.00	0.03±0.003^{g}	0.03±0.003^{g}
0.05	0.05±0.005^{f}	0.08±0.001^{f}
0.10	0.18±0.004^{e}	0.09±0.003^{e}
0.15	0.32±0.013^{d}	0.14±0.003^{d}
0.20	0.39±0.006^{c}	0.24±0.002^{c}
0.25	0.42±0.008^{b}	0.34±0.001^{b}
0.30	0.47±0.009^{a}	0.42±0.003^{a}
F	*	*
CV (%)	0.01	0.01

Notes: Numbers in a column with distinguished letters are significantly different at $p<0.05$ by using Duncan's multiple range test; data were shown by MEAN±SE.

Overall, the extracts of *Rhodotorula sphaerocarpa* 5S1-T and *Rhodotorula sphaerocarpa* 5S4-T both had a reducing capacity with increasing absorbances from 0.05 to 0.3 mg/ml. They were significantly different in terms of the absorbances of various extractive concentrations. These results demonstrate that the reducing capacity of the total carotenoid extract of *Rhodotorula sphaerocarpa* 5S1-T (0.47±0.009) is higher than that of *Rhodotorula sphaerocarpa* 5S4-T (0.42±0.003). It is proved that *Rhodotorula sphaerocarpa* 5S1-T possesses high antioxidant activity. E.P. Cipolatti, et al. (2019) [29] affirmed that by using the ferric reducing antioxidant capacity, the extracted carotenoid of *Rhodotorula mucilaginosa* presented a potent antioxidant compound. M.R.A. Manimala and R. Murugesan (2014) [30] researched the antioxidant activity of carotenoid pigment extracted from *Sporobolomyces* sp. - a genus of yeast as a carotenoid producer - and reported a high efficiency of ferric reducing power for the antioxidant assay with an absorbance of 0.99 at 0.02 mg/ml.

This study discovered two strains of marine yeast falling into the genus *Rhodotorula* as a carotenoid producer. All assays revealed that the total carotenoid extracted from the two strains *Rhodotorula sphaerocarpa* 5S1-T and *Rhodotorula sphaerocarpa* 5S4-T both had good antioxidant activities. Although the two strains belong to the same species *Rhodotorula sphaerocarpa*, the extracts showed different antioxidant abilities. This is evidence to distinguish the subspecies, however, there is a diversity of species in a genus.

Further studies needs to be done to optimise elements such as carbohydrate sources, pH, temperature, and medium to reach the best conditions for producing the carotenoid of *Rhodotorula sphaerocarpa* 5S1-T and *Rhodotorula sphaerocarpa* 5S4-T. It is possible to use carotenoids extracted from these two strains in feed and foods as natural bioactive compounds as well as colorants.

4. Conclusions

Twenty-three samples were isolated from soils collected in the coastal environment of the Kien Giang and Tra Vinh provinces. Based on characteristics of morphology, physiology, biochemistry, and molecular, it is clear that both isolates 5S1-T and 5S4-T belong to the yeast. The ITS rDNA sequence of isolates 5S1-T and 5S4-T evidenced a 100% similarity with *Rhodotorula sphaerocarpa* 5940 and *Rhodotorula sphaerocarpa* 5939. Moreover, the content of total carotenoid extracted from two strains 5S1-T and 5S4-T was determined to be 1.81 and 1.16 mg/l, respectively. Besides, the antioxidant activity of the carotenoid extracts of the two strains was evaluated as a good antioxidant source by testing the DPPH free radical scavenging and ferric reducing antioxidant capacity.

CRediT author statement

Luong Anh Hue: Experimental design, Methodology, Data analysis, and Writing paper; Nguyen Thanh Trung Nhan: Investigation and Data collection; Nguyen Minh Chon: Conceptualization, Review, and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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