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GC-MS profiling of carotenoid pigment produced by *Gordonia terrae*

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Carotenoids are one of the most essential groups of naturally occurring lipid-soluble pigments that display great biological activities, such as antioxidant, anti-inflammatory, and provitamin A activities. Carotenoids are widely applied in various industries due to their advantageous health effects. Microbial carotenoid synthesis, therefore, has attracted increasing attention in recent years. In the present study, halophiles isolated and characterised from the Solar saltern of Mulund, Mumbai, Maharashtra, were determined to be a strain of *G. terrae* based on its 16S rRNA gene sequence. The strain *G. terrae* has the ability to synthesise and accumulate the intracellular pigments, which was identified by gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared (FTIR). Findings suggested that *G. terrae* can actively grow and efficiently synthesise carotenoids. GC-MS results revealed that the pigment sample contains nearly 17 different compounds. 17 recorded compounds are terpenoids and organic pigments. These compounds were recorded for the first time from the Solar saltern of Mulund, Mumbai, Maharashtra. Therefore, the new bacterial strain isolated and characterised from the Solar saltern of Mulund, Mumbai, showed a high potential bioresource for the commercial production of natural carotenoids.

Keywords: Carotenoids, FTIR, GC-MS, Halophiles, Solarsaltern

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Introduction

Natural and synthetic pigments are widely used in foods, cosmetics, medicines, and agriculture products. Currently, there exists an increasing trend of public awareness on adopting measures that are environmentally friendly and that improve human safety[1]. People have shown their aversion to synthetic pigments, therefore natural pigments are preferred by consumers[2]. Carotenoids are the most widespread group of natural pigments[3,4]. Those reddish, orange, or yellowish pigments observed in living microorganisms are mainly carotenoids. Carotenoids naturally exist in photosynthetic organisms as a beneficial fat-soluble pigment[5,6].

Furthermore, natural carotenoids are also found in certain non-photosynthetic organisms, where they protect cells from light and oxygen damage[6]. In addition, carotenoids show antioxidant, antitumor, and antimicrobial properties; therefore, they are widely used in foods, medicines, feeds, and cosmetics fields[7-10]. Currently, natural carotenoids are mainly produced by plants; however, natural carotenoid production through extraction from plants is limited by uncertain seasonal and geographic variability[8]. This leads to research on microbial fermentation for producing natural carotenoids as potential alternatives. Bacterial production of carotenoids has recently come into the spotlight due to their various advantages, such as fast and easy cultivation, higher biomass in shorter periods, no seasonal restrictions, nontoxicity, and efficient extraction process[6,11]. Nowadays, 722 organisms have been described as the source of the 1204 natural carotenoids currently defined in the Carotenoid DataBase (http://carotenoiddb.jp (accessed on updated: September 2020))[12-14]. Bacteria species such as *Micrococcus spp*., *Flavobacterium spp*., *Agrobacterium spp*., *Chromobacterium spp*., *Arthrobacter spp*., *Serratia marcescens*, *Pseudomonas aeruginosa*, *Rheinheimera spp*., *Gordonia alkanivorans* and *Brevibacterium linens*[6,15-18] have been reported for carotenoid production, and studies demonstrate that commercial microbial production of carotenoid is still in the research and development phase. The growing worldwide interest in the development and production of carotenoids from natural sources has boosted researcher's interest in finding new microorganisms

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for naturally derived carotenoids from different sources in the environment.

Carotenoids have gained a reputation not only as nutraceuticals and nutricosmetics but also as biologically active molecules[6]. Most studies on carotenoids are based on their robust antioxidant capabilities[1,3]. There has been a growing interest in the study of carotenoid's influence on eye problems, such as cataracts and age-related macular degeneration, as well as the defence of the retina against exposure to phototoxic light damages[19–22].

In this work, a novel strain with pigment biosynthesis ability was isolated and identified as *G. terrae* from Solar saltern of Mulund, Mumbai, Maharashtra. Gas Chromatography-Mass Spectrometry (GC-MS) is a widely-used analytical tool due to its versatility and precision in both quantitative analysis and identification of unknown compounds. Thus, the structural characteristics of the extracted pigment were explored by Gas chromatography–mass spectrometry (GC-MS) and Fourier transform infrared (FTIR). FTIR-based characterisation of extracted pigment was done to find out the chemical nature of the pigment.

Materials and Methods

Sample collection and isolation of pigment-producing bacteria from solar saltern

The sample was collected from marine Solar slatterns of Mulund (E) (19010'12''N,7207'18''E) Mumbai from different locations around the area. The appropriate salt processing period in these salt pans is about four months. It starts in the middle of February and ends in the first week of June. For this study, samples were collected in May 2018 and May 2019. The sample was identified by Prof. Anupama Pathak, Head, Department of Microbiology, SRTMU, Nanded. They were mixed to get an adequate representation of the local micro-flora. Different media, such as Nutrient agar and Luria Bertani agar with variable concentrations of NaCl, were used for the cultivation of microorganisms.

All the samples were mixed in equal proportions and used to isolate halophiles. Aliquots of 100 µL of composite hyper saline samples were plated on solid media. Plates were observed nearly 14 days of incubation once the microbial growth had been observed. The number of colonies noted and colony characteristics were recorded. Among the different media used for cultivation, the medium that showed the highest diversity and supported faster growth was selected for further investigation[23]. Using the selected medium, the minimum salt requirement for growth was determined by varying NaCl concentration in the range of 0 to 30%. The isolated colonies were purified by successive streaking on Nutrient agar medium containing NaCl. Three potential isolates were selected based on the difference in the colour of colonies. For this, isolates were streaked on Nutrient agar medium, incubated at 37°C for 72 h, and observed for pigment production. Our study observed that once the growth temperature was raised above 37°C, the pigment stopped being produced because above this temperature affects the pigment production. The isolates that showed bright pigmentation were selected. Purified isolates were preserved as 30% glycerol stock at -80°C[24,25].

Selected colonies were sub-cultured on nutrient agar plates with 20% salt and incubated at 37°C. Microscopic and macroscopic features of grown isolates were recorded. Amongst many pigmented colonies, intense orange-coloured fast-growing colonies were selected for further analysis. Isolated halo bacteria was identified as *G. terrae* by biochemical and molecular techniques[24,25].

Production and extraction of microbial pigments

Bacterial isolates were grown in a nutrient agar medium and broth. For large-scale production of pigments, an equal number of cells were inoculated in 1000 mL freshly prepared Nutrient broth medium and incubated in an incubator shaker at a temperature of 37ºC at 180 rpm for 72 hours and visually observed for pigment production. After 72 h, the bacterial culture was centrifugedat 7000 rpm for 30 min. After centrifugation, the supernatant was discarded, and bacterial cell pellets were processed for extraction of pigments. To the cell pellets, 1 mL mixture of acetone and methanol (3:1) was added and vortexed until the cell pellets turned colourless. The cell debris was then discarded, while the supernatant was transferred to a glass Petri plate and dried overnight in an incubator at 37°C. The dried pigment was scrapped out and dissolved in methanol[23,26-28]. This pigment sample was further processed for GC-MS and FTIR analysis.

GC-MS analysis

GC-MS (Shimadzu GC-MS QP2010S) analysis was carried out under the following conditions: carrier gas: helium; column: J&WDB-1 (30 m length, 0.25 mm ID, 0.25 µm thickness); Oven temperature: 50°C

(4 min hold) increased to 280°C at a rate of 10°C/min (10 min hold), MS source temperature: 260°C, Injection Temperature: 220°C, Carrier Gas Flow Rate: 1.0 mL/min, Split ratio: 10:1, Injection mode: Manual, Injection volume: 1.0 µL. For the ionisation of electrons, 70 eV was used. The scan range was 50-500 m/z. The mass of the compounds and the fragments recorded were matched with NIST 11 and Wiley 8 libraries to identify probable compounds present in the sample. PubChem database and other research articles were cross-referred to the structure and activities of the detected compounds[28,29].

Results

In the present study, the pigment sample produced by *G. terrae* was subjected to GC-MS analysis. The chromatograms obtained are presented in Fig. 1. The mass of compounds and fragments recorded were matched with NIST 11 and Wiley 8 libraries for identifying probable compounds, which accounted for a total of 17 compounds present in the tested samples (Table 1). These compounds are 1,6-Anhydro-3,4-dideoxy-β-D-manno-hexapyranose; n-Decanoicacid; 7-Tetracene; n-Undecanoicacid; n-Octanol; n-Hexadecanol; n-Octadec-1-ene; E-14-Hexadecenal; [(Dodecycloxy)methyl)]-oxirane; E-3-Eicosene; 2-methyl-e-7-hexadecene; Pthalicacid,bis(2-ethylhexyl) ester(6Cl,8Cl); 1,6,3,4-Dianhydro-2-deoxy-β-D-lyxo-hexopyranose; Cyclooctacosane and Heptafluorobutanoic acid. Heptadecyl ester (Fig. 1). Among the 17 recorded compounds, certain members belong to the family of terpenoids, which are organic pigments that underwent analysis and characterization through FTIR (Fourier-transform infrared) and GC-MS (Gas Chromatography-Mass Spectrometry). These specific compounds were documented for the first time in samples collected from the Solar Saltern of Mulund, located in Mumbai, Maharashtra. In the current study, GC-MS data showed 7-Tetracene, which is a polycyclic aromatic hydrocarbon. It has the appearance of a pale orange powder.

FTIR Spectroscopic analysis

The FTIR spectrum of pigment produced by *G. terrae* showed the presence of various functional groups of the active components based on the peak values in the FTIR radiation spectrum (Fig. 2). The results of FTIR analysis confirmed the presence of a broad peak at 3300 cm^{-1} indicates the -OH of carboxylic acid functional group and the stretching at 1660-1730 cm^{-1} implies the presences of carbonyl group (>C=O) analogous to carboxylic acid and ketonic group, whereas polycyclic aromatic/aliphatic (>C=C<) stretching appears at 1600-1530 cm^{-1} were also observed in the lower range of frequency.

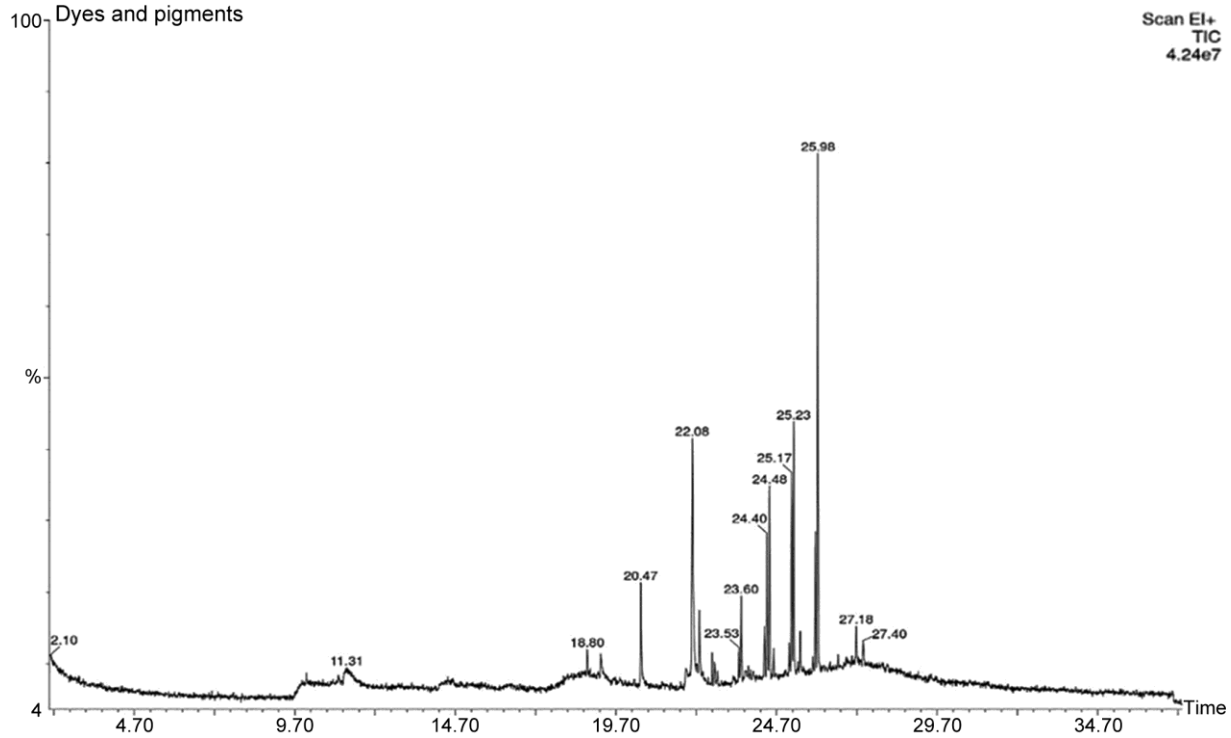

Fig. 1 — Chromatogram of pigment produced by *G. terrae*.

Table 1 — GC-MS analysis of pigment produced by *G. terrae*

Sr. no.	Compound name	RT (mins)	Molecular weight	Area	% Area
1	1,6-Anhydro-3,4-dideoxy-β-D-manno-hexapyranose	18.80	130	110731	2.304
2	1,6-Anhydro-3,4-dideoxy-β-D-manno-hexapyranose	19.23	130	133222	2.773
3	n-Decanoic acid	20.47	172	315817	6.573
4	7-Tetracene	22.08	196	1029322	21.422
5	n-Undecanoic acid	22.30	186	279384	5.814
6	n-Octanol	22.76	128	47732	0.993
7	n-Hexadecanol	22.86	242	35957	0.748
8	n-Octadec-1-ene	23.53	252	69590	1.448
9	E-14-Hexadecenal	23.60	238	131001	2.726
10	[(Dodecycloxy)methyl)]-oxirane	24.32	242	102370	2.130
11	E-3-Eicosene	24.40	280	257596	5.361
12	E-3-Eicosene	24.48	280	348977	7.263
13	2-methyl-e-7-hexadecene	25.17	238	344783	7.175
14	Pthalicacid, bis(2-ethylhexyl)ester(6Cl,8Cl)	25.23	390	433839	9.029
15	1,6,3,4-Dianhydro-2-deoxy-β-D-lyxo-hexopyranose	25.44	128	78578	1.635
16	Cyclooctacosane	25.91	392	226846	4.721
17	Heptafluorobutanoic acid. Heptadecyl ester	25.98	452	859306	17.883

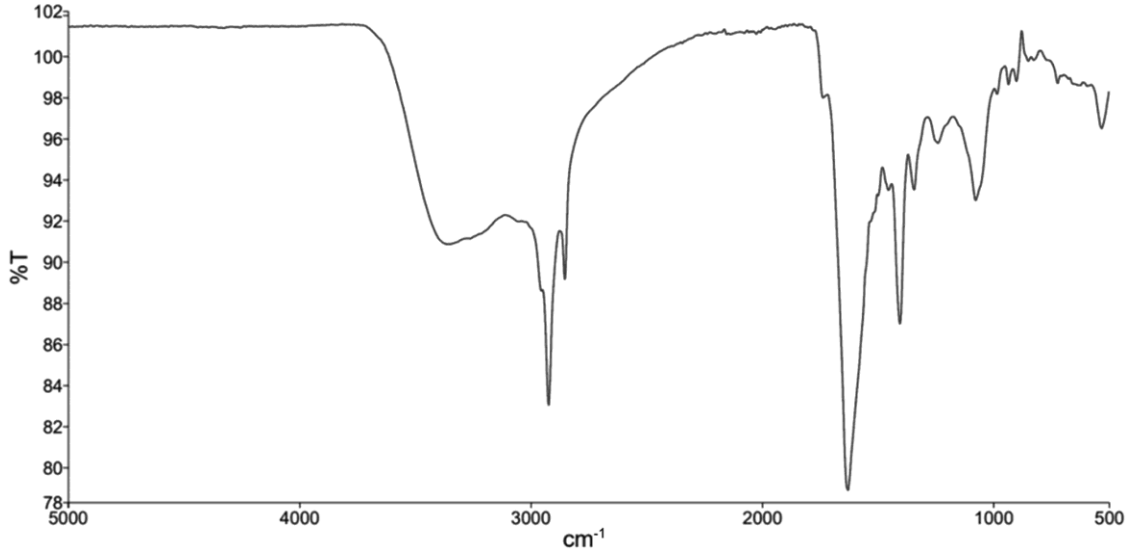

Fig. 2 — FTIR spectrum of pigment produced by *G. terrae*.

However, the stretching corresponding at 1250-1100 cm^{-1} attributes the presence of (-C-O-) functionality, which indicates the presence of ether and alcoholic groups, whereas the stretching observed at lower frequency analogues to heterocyclic compounds (Fig. 2).

Discussion

Solar salterns are known to harbour a high number of taxonomically diverse halophilic organisms. Understanding this ecosystem is, therefore, highly desirable. Salterns originating microorganisms bear biotechnological potential for the production of hydrolytic enzymes, exopolysaccharides, carotenoid pigments etc. Currently, carotenoids are valuable bioactive molecules for several industries, such as chemical, pharmaceutical, food and cosmetics, due to their multiple benefits as natural colourants, antioxidants and vitamin precursors. Hence, the increasing interest in these high-added-value products has led to the search for alternatives, more cost-effective and with better yields, towards their industrial production. Indeed, microbial metabolism offers a promising option for carotenoid production. For the first time, the present investigation reported halophilic bacteria from the Solar saltern of Mulund, Mumbai, producing carotenoid pigments.

In the present study, we have isolated and identified Halophiles from the interconnected multi

pond Solar saltern of Mulund (E.) Mumbai (19°10'12''N,72°57'18''E). From identified isolates, remarkable carotenoid production by *G. terrae* was recorded at pH 7, temperature 30°C and NaCl concentration of 5%. Similarly, a previous study by Aparna *et al.*[25] showed carotenoid-producing Halophiles from the Solar saltern of Mulund, Mumbai, Maharashtra. Extensive studies on various hypersaline environments in different geographical locations have been conducted over the last few decades, as they are known to play a key role in the diversity that has permitted the isolation and taxonomic characterisation of many halophilic species[30,31]. Many other studies have been conducted from different parts of Maharashtra, such as the Unkeshwar hot spring, Bordi Region, and coastal region, Maharashtra[32-37]. Very few studies[23] have been done on the isolation and identification of Halophiles from the Solar saltern of Mulund, Mumbai. Microbiota reported in this work include carotenoid pigments producing Halophiles. The absence of prior studies reporting GC-MS analyses of these isolates underscores the novelty of this research. This study serves as the inaugural exploration into both the production of carotenoids by *G. terrae* and the GC-MS analysis of pigments sourced from the Solar Saltern of Mulund, Mumbai, Maharashtra. Among the 17 documented compounds, noteworthy members pertain to the terpenoids family, and their characterization involved FTIR and GC-MS analyses. This discussion highlights the unique contributions of the current study to the understanding of organic pigments in this specific environment and the potential of the bacterium *G. terrae*, a high carotenoid-producer microorganism.

Conclusion

Solar salterns are known to harbour a high number of taxonomically diverse halophilic organisms. Understanding this ecosystem is, therefore, highly desirable. In the present study, we have investigated for the first time the presence of halophilic bacteria, i.e. *G. terrae*, producing carotenoid pigments and GC-MS profiling from Solar saltern of Mulund, Mumbai, India. This study marks a significant milestone as the first investigation unveiling carotenoid production by *G. terrae* and employing GC-MS analysis to scrutinize pigments from the Solar Saltern of Mulund, Mumbai, Maharashtra. The identification and characterization of terpenoids within the 17 recorded compounds, using FTIR and GC-MS, contribute novel insights into organic pigments in this specific environment. Notably, the absence of subsequent GC-MS analyses by other researchers underscores the pioneering nature of this research, emphasizing its unique contribution to the scientific understanding of these isolates. Microbiota reported in this work have the ability to produce carotenoids, which can be used to produce carotenoids in large amounts.

Conflict of interest

The authors declare no conflict of interest.

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