SHORT COMMUNICATION

Molecular characterization and antioxidant assay of pigment producing bacteria, *Sphingomonas paucimobilis* and *Microbacterium arborescens* isolated from fresh water sediments

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ABSTRACT

This study focuses on isolation of pigment producing bacteria from fresh water sediment. The isolated bacteria were grown in nutrient broth and the maximum absorbance of 2.512 was obtained for the extracted pigment at 500 nm. The effective strains were optimized, pH 11 and temperature 30 °C was found to be more favorable for its maximum growth. The isolates were identified based on their molecular characteristics as *Microbacterium arborescens* and *Sphingomonas paucimobilis*, molecular size of the amplified 16S rRNA gene sequence was found to be approximately 1270 and 765 bp respectively. The antioxidant property of the pigment was analyzed using DPPH and ABTS assay. The IC50 value of *Microbacterium arborescens* was higher in all the three assays in comparison with *Sphingomonas paucimobilis*. The extracted pigment was characterized for the presence of compounds using GC-MS and FTIR analysis to determine the functional groups. As the pigment obtained from *M. arborescens* had shown better antioxidant activity it may be used as colorant in food industrial applications.

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1. Introduction

Pigments have a wide range of applications in various types of industries viz., food, pharmaceutical, cosmetic and textile, with salient features that make these products more striking in appearance. The increased need for safe and natural products for human consumption and awareness in order to conserve the environment has led to an increased research on pigment producing bacterial strains. (Aruldass et al. 2016)

Food colorants are added to enhance the quality and appearance thereby by giving pretty and restoring natural colors that may get lost during the processing and to ensure uniformity within different batch. (Vaclavik and Christian 2014). Most of the safe natural colorants that are used in nutraceuticals, food, feed, cosmetics and pharmaceutical industries are carotenoids. Carotenoids mainly occurs in both prokaryotic and eukaryotic organisms, where they protect the cell from light mediated damage (Britton 1995). Earlier reports show that the natural antioxidants have less toxicity (Chun et al. 2003). The enzymatic and non-enzymatic forms of antioxidants which are found both within and outside the cellular environment. The cell components of our body are amended during oxidative stress conditions, leading to diseased condition. The effect of oxidative stress within the cell can be efficiently stabilized by augmenting cellular defense in form of antioxidants (Pal and Nimse 2006). The resulting oxidative damage is a common cellular event implicated in a broad spectrum of pathological circumstances, including all infectious, inflammatory, neurodegenerative and cardiovascular diseases, cancer and diabetes (Hristova-Avakumova et al. 2017). Moreover, the synthetic antioxidants are leading cause for health hazards, so efforts are made to select natural antioxidants (Lee et al. 2014). Antioxidants that are dietary are proven to be very effective against chronic diseases. Recent research is mainly focused on natural antioxidants from plants and microbes that serves as safer therapeutics (Thenmozhi et al. 2010).

The present study deals with isolation of pigment producing bacteria from fresh water sediments and test its antioxidant potential. To the best of our knowledge there are no previous reports on pigment production from these isolated strains namely Sphingomonas paucimobilis and Microbacterium arborescens. The extracted bacterial pigments were tested for the free radical scavenging activity by DPPH and ABTS assays.

2. Results and discussion

2.1. Isolation and molecular identification of pigment producing bacteria

The present study deals with the isolation of two bacteria from fresh water sediments that were capable in pigment production (Figure S1a,b). These two bacteria were identified based on their morphological and biochemical characteristics (Figure S2a,b; Table S1). The species level identification was confirmed by molecular characterization. The colonies were transferred to the nutrient broth for the pigment production with optimized temperature and pH.
Molecular characterization of bacterial isolates revealed the size of the genomic DNA to be 10,000 bp and that of the amplified product as 1271 bp and 765 bp (Figure S3). Species level identification confirmed the two bacterial isolates as *S. paucimobilis* MH400706 and *M. arborescens* MH400707. Comparison in the genetic similarity of the isolated bacteria was performed by BLAST online tool, with the existing reported genus as shown in phylogenetic tree (Figure S4a,b). A wide range of pigments viz., yellow, orange, and red were produced by *M. purpureus*. Among these pigments the red pigment had higher excitation under suitable conditions (pH 6.0 and temperature 30 °C) (Carvalho et al. 2005).

### 2.2. Optimization of pigment

Our study involved extraction of pigment by methanol and optimization for production at varying pH and temperature (Figure S5a,b). It was found that pH 11 and 30 °C was most favorable condition for maximum pigment production, analyzed by absorbance obtained in UV spectrophotometer (Figure S6). The maximum optical density of 1.621 was attained at pH 11 and 2.512 was obtained at 30 °C showing that *M. arborescens* produced better yield at these optimized conditions (Figure S7a,b). Surekha et al. (2016) reported a yellow pigment producing bacteria named *Micrococcus luteus* strain BAA2 (Gen Bank Accession No. KF550912) that was grown on nutrient agar plates and incubated at 37 °C for 3 days, yellow colored colonies were observed.

### 2.3. Antioxidant assays

The two extracted pigments were tested for free radical scavenging ability by ABTS, DPPH and Reducing power assays. Ascorbic acid was used as a standard for all the antioxidant assays performed. When pigments were tested against DPPH radical a IC50 value of 3.1 mg/ml with maximum scavenging of 56 ± 0.21% was shown by pigment obtained from *S. paucimobilis* and a IC50 value of 4.8 mg/ml and scavenging of 54 ± 0.21% for *M. arborescens*. In ABTS assay, a maximum scavenging activity of 57.1 ± 0.7% at 6 mg/ml with IC50 value of 5.6 mg/ml in pigment obtained from *M. arborescens* compared to *S. paucimobilis* with maximum scavenging of 49.4 ± 1.2% (IC50 value- 4.254 mg/ml). The ascorbic acid had shown a IC50 value of 18 μg/ml against DPPH radical and 22 μg/ml against ABTS radical. (Fig S8a,b,c). The reducing power results revealed maximum absorbance of 0.44 for pigment of *M. arborescens* and 0.32 for pigment of *S. paucimobilis* at 1200 μg/ml concentration, whereas the absorbance of 0.9 was obtained for Ascorbic acid. The present findings revealed that the pigment obtained from *M. arborescens* had shown maximum absorbance and the radical scavenging activity was better compared to the other strain.

### 2.4. Characterization

The pigments were characterized by GC-MS and FTIR analysis, the GC spectrum of pigment extracted from *S. paucimobilis* showed eight compounds phenol,
2,4-bis(1,1-dimethylethyl), 3,7,11,15 –tetramethyl-2-hexadecen-1-ol, N-Hexadecanoic acid, tetradecanoic acid, oleic acid, cholest-8-en-3-ol, 14-methyl (3.beta.,5.alpha) and cyclohexane, 1,1′-(2-tridecyl-1,3-propanediyl)bis and 7-hydroxy-3-(1,1-dimethyl-prop-2-enyl) coumarin (Table S2a). A phenol compound produced by *Vibrio alginolyticus* was proven to have antifouling property (Padmavathi et al. 2014). Similar metabolites were synthesized by their marine Vibrio strain PIGB 184 that strengthens their views on contribution of pyrrolo and phenol derivatives towards observed antioxidant and antibacterial effects.

In the GC MS analysis of pigment from *M. arborescens* revealed a total of ten compounds found in the pigment namely phenol, 3,5-bis(1,1-dimethylethyl), 3,7,11,15-tetramethyl-2-hexadecen-1-ol, eicosanoic acid, octadecanoic acid, tridecanoic acid, n-hexadecanoic acid and 2-propenoic acid, oxybis (methyl-2,1-ethanediyl) ester, T-Butyl cyclopentaneperoxycarboxylate, 7-hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin, 14-heptadecenal compound (Table S2b). In both the pigments a similar compound named phenol, 3, 5-bis (1,1-dimethylethyl), molecular weight-206 was present. Based on the previous reports this compound was responsible for antioxidant activity, so the compound obtained may play a role in free radical scavenging (Figure S9a,b).

In the FTIR analysis of the pigment obtained from *S. paucimobilis* showed strong absorption spectrum at 3414.13 cm⁻¹, 2926.08 cm⁻¹, 2855.89 cm⁻¹, 1631.34 cm⁻¹, 1405.59 cm⁻¹, 111.40 cm⁻¹, 619.43 cm⁻¹ and 538.08 cm⁻¹. The FTIR spectrum of the *M. arborescens* shows strong absorption spectrum at 3420.74 cm⁻¹, 2960.72 cm⁻¹, 2930.34 cm⁻¹, 1632.58 cm⁻¹, 1404.12 cm⁻¹, 1112.44 cm⁻¹, 619.50 cm⁻¹ and 538.36 cm⁻¹. The vibrations of hydroxyl and amino groups formed in the range 3600–2800 cm⁻¹ represents aromatic amino, phenolic and carboxylic functional groups of indolic and pyrrolic. The formation of spectrum between 1750– 1550 cm⁻¹ represents the vibrations of C—O. The bending groups (O—H) of carboxylic and phenolic groups are observed in 1400–1300 cm⁻¹. The spectrum obtained from FTIR indicates strong bands at 3422 cm⁻¹ (Hydroxide group or amino group), 2893 and 2834 cm⁻¹ (Aromatic or benzene group), 1761 cm⁻¹ and 1643 cm⁻¹ showed aliphatic group as shown in Figure S10a,b.

### 3. Conclusion

The present study involves the isolation of pigment producing bacteria from fresh water sediment. Growth of the bacteria was optimized and the radical scavenging activity of the extracted pigments was done, it was further characterized by GC-MS and FTIR to find out the compound responsible for it.

Production of pigment from bacteria and other natural source is one of the emerging fields of research that had its potential industrial applications. From the present findings it is clear that the extracted pigment has antioxidant activity and it can be used in food industry as a colourant.

### Disclosure statement

No potential conflict of interest was reported by the authors.
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