Indian Journal of Geo Marine Sciences
Vol. 54 (12), December 2025, pp. 656-663
DOI: 10.56042/ijms.v54i12.24913

***Research Article***

# Production of water-soluble melanin pigment by marine derived *Penicillium citrinum* 2332 occurring as a sponge endosymbiont

J Manochkumar[a], M B Govinda Rajulu[b], T S Suryanarayanan*[,b], N Thirunavukkarasu[c], M D Kalaiarasan[b,c], S Bharathwaj[b,c] & S Ramamoorthy[a]

[a]School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu – 632 014, India

[b]Vivekananda Institute of Tropical Mycology (VINSTROM), A research Unit of Ramakrishna Mission Vidyapith, Chennai, Tamil Nadu – 600 004, India

[c]PG & Research Department of Botany, Ramakrishna Mission Vivekananda College (University of Madras), Chennai, Tamil Nadu – 600 004, India

*[Email: t_sury2002@yahoo.com]

*Received 4 November 2025; revised 21 November 2025*

Melanin pigments are dark coloured and made of heterogeneous polymers of phenolic and/or indolic monomers and are produced by a wide range of organisms, including fungi. Although melanins are insoluble, pyomelanin is water-soluble and is endowed with antimicrobial, antioxidant, and anti-inflammatory properties. These features, along with their UV-screening ability, enhance the ecological fitness of pyomelanin-producing organisms. Most studies on microbial pyomelanin are focused on bacteria, and there are very few studies on fungal pyomelanin. The present study reports for the first time, that a marine-derived isolate of *Penicillium citrinum* 2332 isolated as an endosymbiont of a marine sponge, produces this technologically useful pigment.

[**Keywords**: Bioactive compounds, Fungal melanin, Marine-derived fungi, Marine sponge, Pyomelanin]

## Introduction

Marine sponges (phylum Porifera) harbour taxonomically and functionally diverse microbial communities, including bacteria, archaea, algae and fungi as symbionts which are adapted to marine conditions, including high salinity, pressure, and nutrient variability[1,2]. Sponges, with their relatively simple body structure, depend on these symbionts for various metabolic functions and defence against pathogens[3]. Most of the studies on symbionts of marine sponges focus on their bacterial associates, and current knowledge of their fungal symbionts is rudimentary[4]. Recent studies show that sponge-associated fungi, due to their unique ecological niche and potential for production of novel bioactive compounds, are of significant scientific and industrial importance[5,6]. The current study on fungal endosymbionts of sponges of the Lakshadweep Archipelago identified a marine-derived *Penicillium citrinum* that produces water-soluble melanin (pyomelanin). This pigment plays many ecological roles and has many technological uses.

## Materials and Methods

### Identification of the endosymbiotic fungus

Current study isolated a reddish-brown pigment-producing endosymbiotic fungus from an unidentified marine sponge (Fig. 1) collected from Kavaratti island (10°33'35.09" N; 72°37'28.28" E) of the Lakshadweep archipelago, India. The identity of the sponge could not be ascertained because key features, such as its morphology and skeletal makeup (spicules), were not clear. Colony and conidial ontogeny characteristics[7], as well as molecular methods, were used for identifying the fungus. The extraction of genomic DNA from the fungus and PCR amplification of the ITS rRNA region were done following the method of Sawmya *et al*.[8]. The sequence obtained has been submitted to GenBank under the accession number PP033985, and the fungal strain has been deposited in the Microbial Type Culture Collection (MTCC), Chandigarh, India (accession number MTCC 13857).

### Evolutionary analysis by the maximum likelihood method

The evolutionary distance was estimated using the maximum-likelihood method and the Tamura-Nei



Fig. 1 — Sponge species (unidentified host) from which *P. citrinum* was isolated

model[9], and the best tree is shown. Values above the branches indicate the percentage of trees with the same taxa clustered together, while the number of substitutions per site was used to calculate branch lengths. A total of 46 sequences were used to build this tree, with a total of 386 positions in the final dataset. The evolutionary analyses were carried out using MEGA11[(ref. 10)].

#### Test for salt tolerance

The fungus was inoculated in 100 mL potato dextrose liquid medium made with sterile distilled water and amended with different concentrations of NaCl (0, 1.5, 3.5, or 7.0 %) and incubated as static cultures at 26±2 °C. After ten days, the mycelium was filtered, dried in a hot-air oven, and the dry weight was measured.

#### Extraction of pyomelanin

Pyomelanin was extracted following the method of Suryanarayanan *et al.*[11]. The fungus was grown in liquid Czapek Dox (CD) medium amended with XAD™ (DuPont) resin beads to adsorb secreted secondary metabolites that could interfere with the pigment analysis. After 30 days of growth, the reddish-brown culture medium was filtered and centrifuged, and the resultant mycelium-free culture filtrate was lyophilised and used for pigment analysis.

#### Solubility test

The solubility of the lyophilised pigment in organic solvents, including ethanol, ethyl acetate, hexane, petroleum ether, acetone, NaOH, KOH and HCl, was tested. Furthermore, its reaction with $FeCl_3$ and $H_2O_2$ was tested.

#### UV, FTIR, EPR and proton NMR tests

The lyophilised pigment was dissolved in 1 mL of methanol, and its ultraviolet-visible spectrum was recorded in the range of 200–800 nm using UV-Vis Spectrophotometer[12]. For the identification and characterisation of functional groups, the Fourier Transform Infrared (FTIR) spectrum of the pigment (range of 500 to 4000 $cm^{-1}$) was obtained[13]. An Electron Paramagnetic Resonance (EPR) spectrum of the lyophilised pigment was obtained using a JEOL Model JES FA200 model spectrometer. The parameters for EPR were X-band frequency: 8.75–9.65 GHz, sensitivity: 7×109 spins, resolution: 2.35 µT, and liquid nitrogen temperature: 77 K. For $^1H$ NMR spectroscopy, the sample was dissolved in deuterated methanol and read using a Bruker NMR Spectrometer (Avance III-400MHz).

#### Treatment with melanin synthesis inhibitors

The fungus was grown in CD medium (100 mL) and amended separately with different melanin synthesis inhibitors. Tricyclazole (DHN melanin inhibitor) was added in concentrations of 1, 10, 100 or 1000 µg, and Kojic acid (DOPA melanin inhibitor) was added in concentrations of 1, 10, 100 or 1000 µg[(ref. 14)]. NTBC (Pyomelanin inhibitor) was added in concentrations of 125, 250, 500, or 1000 µM[(refs. 11,15)]. The cultures were incubated for 30 days and checked periodically for inhibition of pigment production. The fungus cultured in an inhibitor-free medium served as a control. All experiments were performed in triplicate and were repeated thrice.

#### Treatment with L-tyrosine

The fungus was grown in CD medium supplemented with 0.5 % L-tyrosine for 30 days at 26 °C[(ref. 16)]. Cultures grown in CD medium without tyrosine served as control[17].

## Results

#### Identity of the fungus

The morphological features of the fungus were consistent with those of *Penicillium citrinum*. The conidiophores were erect, smooth-walled, and terminated in a brush-like penicillus comprising a primary stalk with metulae and flask-shaped phialides arranged in a verticillate pattern. Long, unbranched chains of globose grey-green conidia (approx. 2 µm

each) arose from the phialides (Fig. 2). The identity of the fungus was confirmed by ITS sequencing (Fig. 3). The sequence showed 100 % match and 100 % query coverage with sequences of *Penicillium citrinum* (Accession No. OR512364), *P. citrinum* (endophytic strain Accession No. OP485654), and *P. citrinum* (endophytic strain Accession No. KU059850) available in the GenBank. The obtained ITS1-5.8S-ITS2 sequence fragment was aligned with 46 nucleotide sequences of *Penicillium* sp. and *Talaromyces* sp. available in the GenBank database. The sequences obtained were aligned using ClustalW[18] with default settings, and the aligned sequences were then manually adjusted for the analysis. The evolutionary relationships among the datasets were inferred using the maximum-likelihood methods[9] in MEGA version 11[(ref. 10)]. Branch support was evaluated through bootstrap analysis with 1000 replicates[19]. *Talaromyces verruculosus* was used as an outgroup. The phylogenetic tree showed that the obtained isolate formed a tight clade with *P. citrinum* isolates. Based on molecular and morphological analyses, the fungal isolate was identified as *Penicillium citrinum*. The fungus grew equally well in NaCl-amended medium and could tolerate a salt concentration of 7 %, which is twice that of normal seawater (Fig. 4). This proved that it was a marine-derived fungus adapted to grow both in non-saline and saline environments and not an obligate marine form which can grow only in the presence of salt.

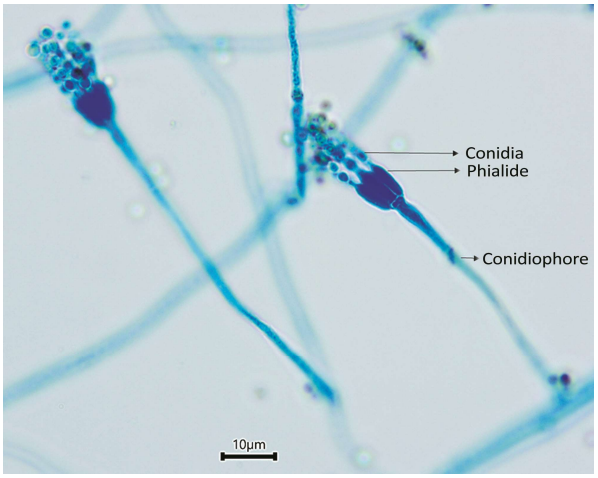



Fig. 2 — Conidia and conidiophores of *P. citrinum* 2332

**Production of pigment and its solubility in different solvents**

When grown in CD agar or liquid medium, the fungus produced a reddish-brown pigment (Fig. 5). The pigment was visible from around the 15th day of

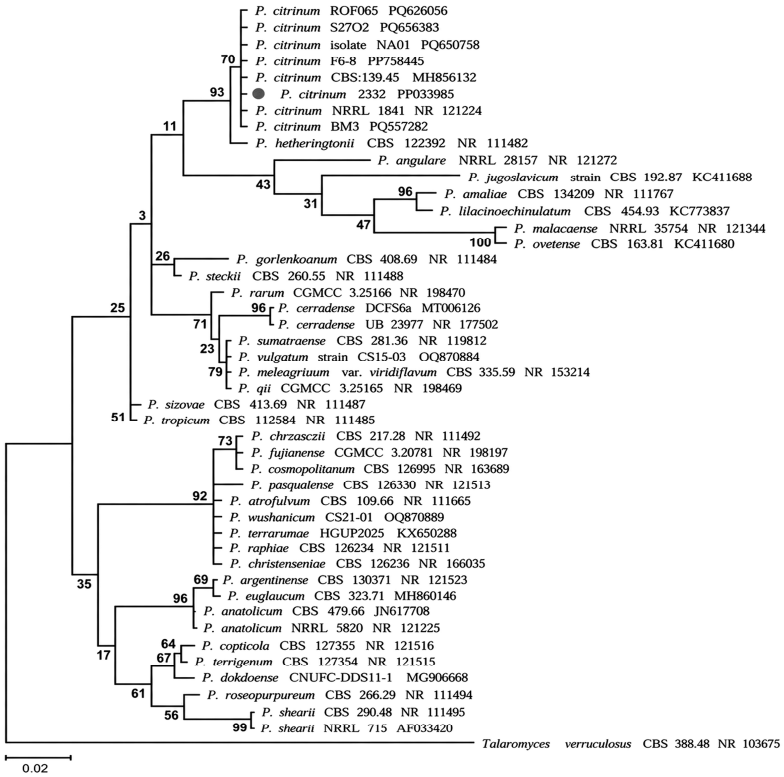



Fig. 3 — Phylogenetic tree constructed using the maximum-likelihood approach showing the highest log likelihood tree for *P. citrinum* 2332

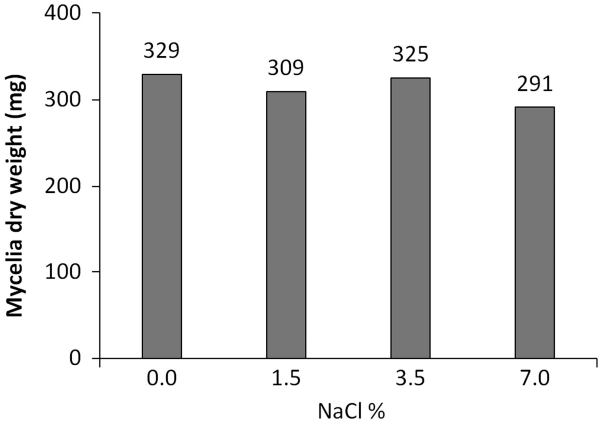



Fig. 4 — Effect of NaCl-mediated growth medium on growth of *P. citrinum* 2332

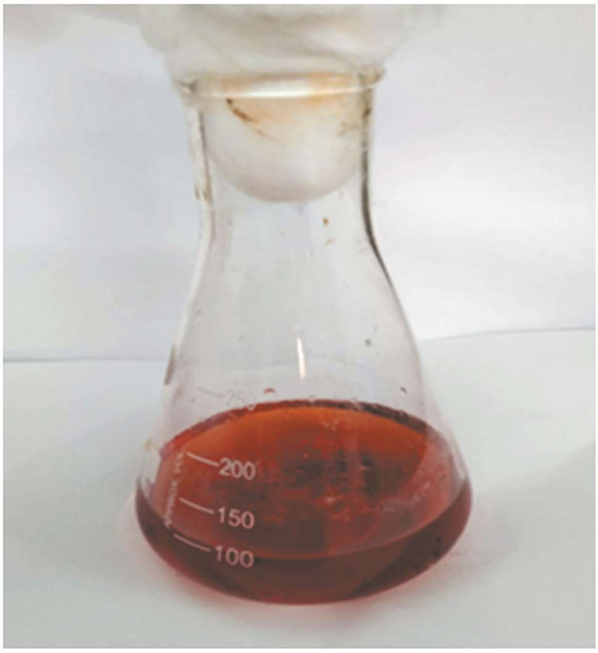



Fig. 5 — 30-day culture of *P. citrinum* showing excreted pigment

incubation. It was extracted after 30 days, since visibly more pigment was present at this time of incubation. The pigment was insoluble in ethanol, acetone, hexane, ethyl acetate, and petroleum ether[20]. However, it was soluble in 1M NaOH, 1M KOH, and precipitated with 3N HCl[21,22], producing a brown precipitate when exposed to polyphenols with $FeCl_3$ (1 % w/v). It did not decolorise in the presence of $H_2O_2$, indicating that it resists oxidative stress[15,20,23].

#### Spectral characteristics of the excreted pigment

The pigment showed maximum absorption at 225 nm in the UV region, which is the characteristic absorbance peak for natural fungal melanin[24]. It decreased progressively as the wavelength increased, such that a log of the absorbance plotted against wavelength was a linear curve with a negative slope[25] (slope = - 0.0049) (Fig. 6a).

The FTIR spectrum of the pigment (Fig. 6b) was typical of melanin with absorption bands ranging from 3600–2900 $cm^{-1}$ and 1670–1600 $cm^{-1}$. The strong absorption between 3600–2900 $cm^{-1}$ is due to -OH and -NH groups of amines, carboxylic acid, phenolic and aromatic amino groups present in the indolic and pyrrolic structures[13,26]. The band between 1670 and 1600 $cm^{-1}$ is attributed to aromatic C=C and C=O stretches of carboxylic groups, which are found in melanins[24]. The absorption between 1600–1500 $cm^{-1}$ and 1400–1300 $cm^{-1}$ was indicative of pyomelanin[27,28]. An electron paramagnetic resonance spectrum (Fig. 6c) showed a peak at 328 milliTesla (3280 Gauss), indicating the presence of stable free radicals, which is characteristic of melanins[29]. A nuclear magnetic resonance spectrum showed resonances between 6.0 and 8.0 ppm (Fig. 6d), indicating the presence of aromatic groups, and between 6.6 and 7.1 ppm due to the presence of C=C. The resonances seen between 0.83 and 4.23 are characteristic of pyomelanin[13].

#### Effect of melanin synthesis inhibitors

The NTBC herbicide (1000 μM), a specific inhibitor of pyomelanin[15,30], inhibited pigment production, thus confirming that it was pyomelanin. Kojic acid (inhibitor of DOPA melanin) or tricyclazole (inhibitor of DHN melanin) did not inhibit the formation of the pigment (Fig. 7).

#### Effect of tyrosine on pigment production

Although the fungus formed the pigment immediately after isolation in different nutrient media, pigment formation was not observed after a few subcultures. However, pigment formation was restored by adding L-tyrosine (0.5 %) to the growth medium.

## Discussion

The study reports the occurrence of *P. citrinum* as an endosymbiont of a marine sponge and its production of the water-soluble melanin pigment (pyomelanin). Pyomelanin, a nitrogen-free, water-soluble melanin, is produced by many bacteria[22]; however, very few fungi have been studied for pyomelanin production. These include *Aspergillus fumigatus*, *A. kawachii*, *Madurella mycetomatis*, *Penicillium chrysogenum* and *Yarrowia lipolytica*[31],

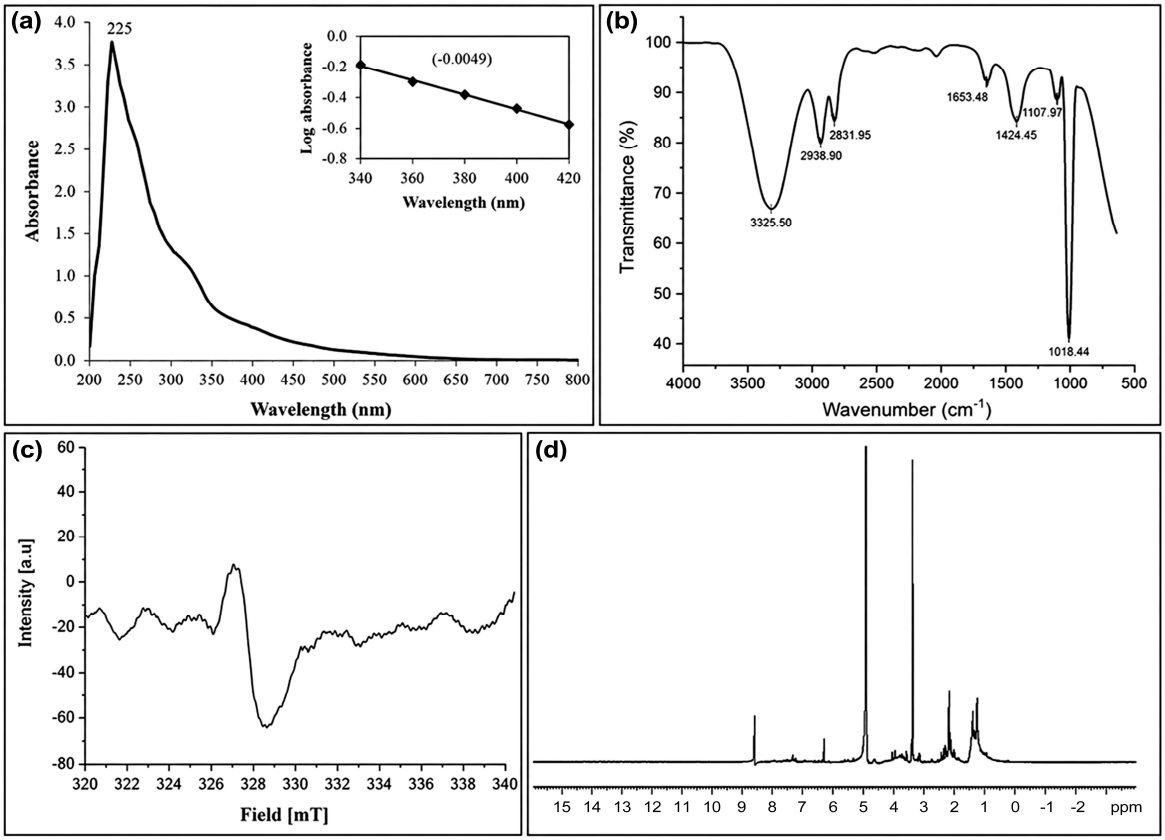



Fig. 6 — Characterisation of the pigment: a) UV-visible spectrum of the pigment and a plot of log of its optical density against wavelength; b) FT-IR spectrum of the pigment; c) EPR spectrum of the pigment; and d) $^{1}$H NMR spectrum of the pigment

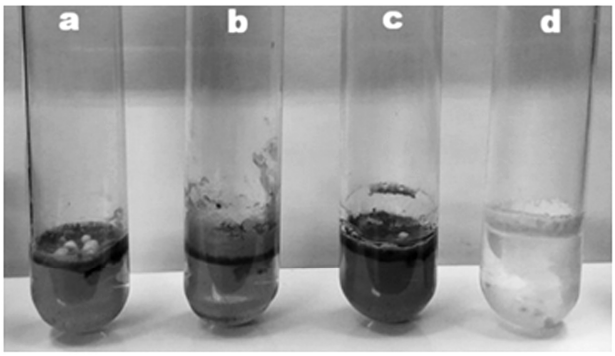



Fig. 7 — Effect of different melanin synthesis inhibitors on pigment production: a) Control without inhibitor, b) Kojic acid 1000 µg, c) Tricyclazole 1000 µg, and d) NTBC 500 µM

*Aspergillus niger*[28,32], *Inonotus hispidus*[33], *Amorosia littoralis*[11], and *Lomentospora prolificans*[34].

The physical and chemical characteristics of the pigment excreted by *P. citrinum* 2332 were similar to those of pyomelanin. The need for L-tyrosine in pigment production indicated that it could be pyomelanin, since pyomelanin formation requires this amino acid[35,36]. Several other features of the pigment confirmed that it is pyomelanin. The maximum absorption of the pigment in the UV region (225 nm) was similar to that of fungal melanin[24]. This negative slope value was within the range reported for fungal melanins (- 0.0015 and - 0.2646)[14,28,37], indicating that the pigment was melanin. FTIR spectrum, which is used for identifying the chemical groups of melanin pigments[34], was similar to the ones reported for pyomelanin of fungi *Amorosia littoralis*[11], *Aspergillus niger*; it was also similar to that of synthetic pyomelanin[28]. The EPR and NMR spectra of the pigment were consistent with pyomelanin[13].

Fungi produce different types of melanins including DHN-melanin, Pyomelanin, DOPA melanin, GBH melanin, PAP melanin, and Catechol melanin. Since the physical and spectral properties of all melanins are similar, the confirmatory method for identifying the type of melanin is the use of inhibitors that specifically inhibit the synthesis of different melanins[34,36,38]. In this study, pigment formation by *P. citrinum* 2332 was inhibited only by a specific inhibitor of pyomelanin synthesis, NTBC {2-[2-nitro-4-(trifluoromethyl)benzoyl]-1,3-cyclohexanedione} and not by inhibitors of DHN and DOPA melanin,

again confirming that the pigment was pyomelanin[20,39].

Pyomelanin is a product of L-tyrosine catabolism. Tyrosine is converted to acetoacetate and fumarate with the formation of 4-hydroxyphenylpyruvate, which is converted to homogentisate (HGA) by 4-hydroxyphenylpyruvate dioxygenase (Hpd). HGA is finally converted to fumarate and acetoacetate by the enzyme homogentisate 1,2-dioxygenase (Hgd). Hyperproduction of HGA or mutations resulting in the absence or dysfunction of Hgd lead to the accumulation of HGA, which is excreted by the organism. The excreted HGA undergoes autoxidation and self-polymerisation to form pyomelanin pigment[40,41]. Pyomelanin increases the competitive ability of the producer organism by scavenging free radicals, binding to various drugs, resisting radiation and reactive oxygen species[15], screening heat[42], enhancing its virulence[43], and being antibacterial and antifungal in nature[44,45]. Furthermore, due to its relatively low molecular weight, it attaches to cell walls and protects fungi from external stress[35]. It is also resistant to microbial enzymes[20]. With such properties, pyomelanin could enhance *P. citrinum's* competitive ability to survive as an endosymbiont in a microbe-rich sponge and as a successful nosocomial human pathogen.

*Penicillium citrinum*, especially when associated with the marine environment, is a prolific producer of several metabolites[46-48]; it is also an opportunistic pathogen in humans[49]. Since pyomelanin is known to protect pathogenic fungi from antifungals[35], the production of pyomelanin could influence their pathogenicity[50]. If this trait is expressed by *P. citrinum* in niches other than the marine niche, it would partly explain the extraordinary ecological success of this fungus, which is one of the most commonly encountered microbes in many habitats. Pyomelanin has attracted attention for its diverse biotechnological uses[20,28]. Since only a few fungi have been explored for this pigment, current study emphasises the need to screen fungi from different ecological niches to maximise pyomelanin's technological uses in industry, healthcare, environmental applications, and energy[51].

## Conclusion

This study demonstrates the production of water-soluble pyomelanin by *P. citrinum* 2332 isolated as an endosymbiont of a marine sponge. Pyomelanin is recognised for its free radical scavenging, antimicrobial, UV screening, antioxidant, and anti-inflammatory properties, and its potential for pharmaceutical applications. The findings of the present study emphasise the significance of exploring fungi from less-studied habitats, such as the marine environment, to broaden our knowledge of pyomelanin produced by fungi and the scope of its uses.

## Acknowledgements

SR thanks the management of Vellore Institute of Technology for their constant support. TSS thanks Swami Dhyanagamyananda, Secretary, Ramakrishna Mission Vidyapith, Chennai, for providing facilities. MBG thanks the Anusandhan National Research Foundation (formerly Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India for the grant of a research project (F. No. CRG/2021/001117). We thank Prof. T. S. Murali, Manipal School of Life Sciences (MAHE), Manipal, Karnataka, India, for molecular identification of the fungus.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

JM, MBGR, SB, NT & MDK: Analytical work, manuscript writing, and preparation of figures. TSS & SR: Conception and designing of the study. JM, MBGR, SB, NT, MDK, TSS & SR: Data acquisition and interpretation.

## References

1 Brinkmann C M, Marker A & Kurtböke D İ, An overview on marine sponge-symbiotic bacteria as unexhausted sources for natural product discovery, *Diversity*, 9 (4) (2017) p. 40. https://doi.org/10.3390/d9040040

2 Carrier T J, Maldonado M, Schmittmann L, Pita L, Bosch T C, *et al.*, Symbiont transmission in marine sponges: reproduction, development, and metamorphosis, *BMC Biol*, 20 (1) (2022) p. 100. https://doi.org/10.1186/s12915-022-01291-6

3 Patel D, Pritee C & Sarika S M, Host–microbial symbiotic relationships in sponges, In: *Microbial Symbionts*, edited by Dharumadurai D, (Elsevier, Amsterdam (NL)), 2023, pp. 681–689. https://doi.org/10.1016/B978-0-323-99334-0.00008-6

4 Pérez-Llano Y, Yarzábal Rodríguez L A, Martínez-Romero E, Dobson A D W, Gunde-Cimerman N, *et al.*, From friends to foes: fungi could be emerging marine sponge pathogens under global change scenarios, *Front Microbiol*, 14 (2023) p. 1213340. https://doi.org/10.3389/fmicb.2023.1213340

5 Giddings L A & Newman D J, Extremophilic fungi from marine environments: underexplored sources of antitumor,

anti-infective and other biologically active agents, *Mar Drugs*, 20 (1) (2022) p. 62. https://doi.org/10.3390/md20010062

6 Gao L W & Zhang P, An update on chemistry and bioactivities of secondary metabolites from the marine algal-derived endophytic fungi, *Phytochem Rev*, 22 (3) (2023) p. 587–614. https://doi.org/10.1007/s11101-022-09852-x

7 Cole G T & Kendrick B, Conidium ontogeny in hyphomycetes. The phialides of *Phialophora*, *Penicillium*, and *Ceratocystis*, *Can J Bot*, 47 (5) (2011) p. 779–789. https://doi.org/10.1139/b69-112

8 Sawmya K, Vasudevan T G & Murali T S, Fungal endophytes from two orchid species pointer towards organ specificity, *Czech Mycol*, 65 (1) (2013) 89–101.

9 Tamura K & Nei M, Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, *Mol Biol Evol*, 10 (3) (1993) 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

10 Tamura K, Stecher G & Kumar S, MEGA 11: Molecular Evolutionary Genetics Analysis version 11, *Mol Biol Evol*, 38 (7) (2021) 3022–3027. https://doi.org/10.1093/molbev/msab120

11 Suryanarayanan T S, Govindarajulu M B, Murali T S, Ravishankar J P, Bharathwaj S, *et al.*, *Amorosia littoralis* Mantle & D. Hawksw. survives as root endophyte of mangroves and produces water soluble melanin pigment, *Fungal Biol*, 128 (5) (2024) 1954–1959. https://doi.org/10.1016/j.funbio.2024.06.001

12 Bayram S, Dengiz C, Gerçek Y C, Cetin I & Topcul M R, Bioproduction, structure elucidation and *in vitro* antiproliferative effect of eumelanin pigment from *Streptomyces parvus* BSB49, *Arch Microbiol*, 202 (9) (2020) 2401–2409. https://doi.org/10.1007/s00203-020-01956-2

13 Bayram S, Production, purification, and characterization of *Streptomyces* sp. strain MPPS2 extracellular pyomelanin pigment, *Arch Microbiol*, 203 (7) (2021) 4419–4426. https://doi.org/10.1007/s00203-021-02437-w

14 Suryanarayanan T S, Ravishankar J P, Venkatesan G & Murali T S, Characterization of the melanin pigment of a cosmopolitan fungal endophyte, *Mycol Res*, 108 (8) (2004) 974–978. https://doi.org/10.1017/S0953756204000619

15 Ketelboeter L M, Potharla V Y & Bardy S L, NTBC treatment of the pyomelanogenic *Pseudomonas aeruginosa* clinical isolate PA1111 inhibits pigment production and increases sensitivity to oxidative stress, *Curr Microbiol*, 69 (3) (2014) 343–348. https://doi.org/10.1007/s00284-014-0593-9

16 Jalmi P, Bodke P, Wahidullah S & Raghukumar S, The fungus *Gliocephalotrichum simplex* as a source of abundant, extracellular melanin for biotechnological applications, *World J Microbiol Biotechnol*, 28 (2) (2012) 505–512. https://doi.org/10.1007/s11274-011-0841-0

17 Almeida-Paes R, Frases S, Araújo G D S, de Oliveira M M E, Gerfen G J, *et al.*, Biosynthesis and functions of a melanoid pigment produced by species of the *Sporothrix* complex in the presence of L-tyrosine, *Appl Environ Microbiol*, 78 (24) (2012) 8623–8630. https://doi.org/10.1128/AEM.02414-12

18 Thompson J D, Higgins D G & Gibson T J, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, *Nucleic Acids Res*, 22 (22) (1994) 4673–4680. https://doi.org/10.1093/nar/22.22.4673

19 Felsenstein J, Confidence limits on phylogenies: an approach using the bootstrap, *Evolution*, 39 (4) (1985) 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

20 Lorquin F, Piccerelle P, Orneto C, Robin M & Lorquin J, New insights and advances on pyomelanin production: from microbial synthesis to applications, *J Ind Microbiol Biotechnol*, 49 (4) (2022) p. kuac013. https://doi.org/10.1093/jimb/kuac013

21 Noman A E, Al-Barha N S & Chen F, Characterization of physicochemical properties of melanin produced by *Gluconobacter oxydans* FBFS 97, *Fermentation*, 8 (11) (2022) p. 574. https://doi.org/10.3390/fermentation8110574

22 Song W, Yang H, Liu S, Yu H, Li D, *et al.*, Melanin: insights into structure, analysis, and biological activities for future development, *J Mater Chem B*, 11 (32) (2023) 7528–7543. https://doi.org/10.1039/d3tb01132a

23 Ahmad S, Lee S Y, Kong H G, Jo E J, Choi H K, *et al.*, Genetic determinants for pyomelanin production and its protective effect against oxidative stress in *Ralstonia solanacearum*, *PLoS One*, 11 (8) (2016) p. e0160845. https://doi.org/10.1371/journal.pone.0160845

24 Medina-Armijo C, Yousef I, Berná A, Puerta A, Esteve-Núñez A, *et al.*, Characterization of melanin from *Exophiala mesophila* with the prospect of potential biotechnological applications, *Front Fungal Biol*, 5 (2024) p. 1390724. https://doi.org/10.3389/ffunb.2024.1390724

25 Bell A A & Wheeler M H, Biosynthesis and functions of fungal melanins, *Annu Rev Phytopathol*, 24 (1) (1986) p. 411–451.

26 Ribera J, Panzarasa G, Stobbe A, Osypova A, Rupper P, *et al.*, Scalable biosynthesis of melanin by the basidiomycete *Armillaria cepistipes*, *J Agric Food Chem*, 67 (1) (2018) 132–139. https://doi.org/10.1021/acs.jafc.8b05071

27 Li Y, Ye Z, Lu P & Lu L, Pyomelanin produced by *Streptomyces* sp. ZL-24 and its protective effects against SH-SY5Y cell injury induced by hydrogen peroxide, *Sci Rep*, 11 (1) (2021) p. 16649. https://doi.org/10.1038/s41598-021-94598-3

28 Koch S M, Freidank-Pohl C, Siontas O, Cortesao M, Mota A, *et al.*, *Aspergillus niger* as a cell factory for the production of pyomelanin, a molecule with UV-C radiation shielding activity, *Front Microbiol*, 14 (2023) p. 1233740. https://doi.org/10.3389/fmicb.2023.1233740

29 Frases S, Salazar A, Dadachova E & Casadevall A, *Cryptococcus neoformans* can utilize the bacterial melanin precursor homogentisic acid for fungal melanogenesis, *Appl Environ Microbiol*, 73 (2) (2007) p. 615–621. https://doi.org/10.1128/AEM.01947-06

30 Ketelboeter L M & Bardy S L, Methods to inhibit bacterial pyomelanin production and determine the corresponding increase in sensitivity to oxidative stress, *J Vis Exp*, 102 (2015) p. 53105. https://dx.doi.org/10.3791/53105

31 Almeida-Paes R, Nosanchuk J D & Zancope-Oliveira R M, Fungal melanins: Biosynthesis and biological functions, In: *Melanin: biosynthesis, functions, and health effects*, edited by Ma X-P & Sun X-X, (Nova Science Publishers, New York, NY, USA), 2012, pp. 77–107. https://doi.org/10.1146/annurev.py.24.090186.002211

32 Keller S, Macheleidt J, Scherlach K, Schmaler-Ripcke J, Jacobsen I D, *et al.*, Pyomelanin formation in *Aspergillus fumigatus* requires HmgX and the transcriptional activator HmgR but is dispensable for virulence, *PLoS One*, 6 (10) (2011) p. e26604. https://doi.org/10.1371/journal.pone.0026604

33 Li X, Wu W, Zhang F, Hu X, Yuan Y, *et al*., Differences between water-soluble and water-insoluble melanin derived from *Inonotus hispidus* mushroom, *Food Chem*, 16 (2022) p. 100498. https://doi.org/10.1016/j.fochx.2022.100498

34 Liporagi-Lopes L C, Chrissian C, Kacirani A, Camacho E, Stark R E, *et al.*, New insights into the melanin structure of *Lomentospora prolificans*, *BioRxiv*, (2024) (*Preprint*). https://doi.org/10.1101/2024.11.01.621558

35 Almeida-Paes R, Almeida-Silva F, Pinto G C M, Almeida M A, Muniz M M, *et al*., L-tyrosine induces the production of a pyomelanin-like pigment by the parasitic yeast-form of *Histoplasma capsulatum*, *Med Mycol*, 56 (4) (2018) p. 506–509. https://doi.org/10.1093/mmy/myx068

36 Rangel-Montoya E A, Paolinelli M, Rolshausen P & Hernandez-Martinez R, The role of melanin in the grapevine trunk disease pathogen *Lasiodiplodia gilanensis*, *Phytopathol Mediterr*, 59 (3) (2020) p. 549–564. https://doi.org/10.14601/Phyto-11685

37 Pralea I E, Moldovan R C, Petrache A M, Ilieș M, Hegheș S C, *et al.*, From extraction to advanced analytical methods: the challenges of melanin analysis, *Int J Mol Sci*, 20 (16) (2019) p. 3943. https://doi.org/10.3390/ijms20163943

38 Diaz Appella M N, Kolender A, Oppezzo O J, López N I & Tribelli P M, The structural complexity of pyomelanin impacts UV shielding in Pseudomonas species with different lifestyles, *FEBS Lett*, 598 (21) (2024) 2702–2716. https://doi.org/10.1002/1873-3468.15000

39 Schmaler-Ripcke J, Sugareva V, Gebhardt P, Winkler R, Kniemeyer O, *et al*., Production of pyomelanin, a second type of melanin, via the tyrosine degradation pathway in *Aspergillus fumigatus*, *Appl Environ Microbiol*, 75 (2) (2009) 493–503. https://doi.org/10.1128/aem.02077-08

40 Kotob S I, Coon S L, Quintero E J & Weiner R M, Homogentisic acid is the primary precursor of melanin synthesis in *Vibrio cholerae*, a *Hyphomonas* strain, and *Shewanella colwelliana*, *Appl Environ Microbiol*, 61 (4) (1995) 1620–1622. https://doi.org/10.1128/aem.61.4.1620-1622.1995

41 Rodríguez-Rojas A, Mena A, Martín S, Borrell N, Oliver A, *et al.*, Inactivation of the *hmgA* gene of *Pseudomonas aeruginosa* leads to pyomelanin hyperproduction, stress resistance and increased persistence in chronic lung infection, *Microbiology*, 155 (4) (2009) 1050–1057. https://doi.org/10.1099/mic.0.024745-0

42 Zeng Z, Cai X, Wang P, Guo Y, Liu X, *et al*., Biofilm formation and heat stress induce pyomelanin production in deep-sea *Pseudoalteromonas* sp. SM9913, *Front Microbiol*, 8 (2017) p. 1822. https://doi.org/10.3389/fmicb.2017.01822

43 Valeru S P, Rompikuntal P K, Ishikawa T, Vaitkevicius K, Sjöling A, *et al*., Role of melanin pigment in expression of *Vibrio cholerae* virulence factors, *Infect Immun*, 77 (3) (2009) 935–942. https://doi.org/10.1128/iai.00929-08

44 Nosanchuk J D & Casadevall A, The contribution of melanin to microbial pathogenesis, *Cell Microbiol*, 5 (4) (2003) p. 203–223. https://doi.org/10.1046/j.1462-5814.2003.00268.x

45 Abdul-Hussien Z R & Atia S S, Antimicrobial effect of pyomelanin extracted from *Pseudomonas aeruginosa*, *Int J Dev Res*, 7 (2017) p. 8692.

46 Wang M L, Lu C H, Xu Q Y, Song S Y, Hu Z Y, *et al.*, Four new citrinin derivatives, from a marine-derived *Penicillium* sp. fungal strain, *Molecules*, 18 (5) (2013) 5723–5735. https://doi.org/10.3390/molecules18055723

47 Yang S Q, Li X M, Li X, Li H L, Meng L H, *et al.*, New citrinin analogues produced by coculture of the marine algal-derived endophytic fungal strains *Aspergillus sydowii* EN-534 and *Penicillium citrinum* EN-535, *Phytochem Lett*, 25 (2018) 191–195. https://doi.org/10.1016/j.phytol.2018.04.023

48 Sabdaningsih A, Liu Y, Mettal U, Heep J, Riyanti Wang L, *et al*., A new citrinin derivative from the Indonesian marine sponge-associated fungus *Penicillium citrinum*, *Mar Drugs*, 18 (4) (2020) p. 227. https://doi.org/10.3390/md18040227

49 Mok T, Koehler A P, Yu M Y, Ellis D H, Johnson P J, *et al.*, Fatal *Penicillium citrinum* pneumonia with pericarditis in a patient with acute leukemia, *J Clin Microbiol*, 35 (10) (1997) 2654–2656. https://doi.org/10.1128/jcm.35.10.2654-2656.1997

50 Fernandes C, Mota M, Barros L, Dias M I, Ferreira I C F R, *et al*., Pyomelanin synthesis in *Alternaria alternata* inhibits DHN-melanin synthesis and decreases cell wall chitin content and thickness, *Front Microbiol*, 12 (2021) p. 691433. https://doi.org/10.3389/fmicb.2021.691433

51 Quin Y & Xia Y, Melanin in fungi: advances in structure, biosynthesis, regulation, and metabolic engineering, *Microbial Cell Fact*, 23 (1) (2024) p. 334. https://doi.org/10.1186/s12934-024-02614-8