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Research Article

Identification and Growth Optimization of *Desertifilum* sp. PSL17 Isolated from Perambalur Lake, Tamil Nadu, India

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Abstract

Cyanobacteria are photosynthetic microorganisms that grow in freshwater and marine water environments which are known for their versatile properties useful in various fields viz. agriculture, medicine and biotechnology industries. In the present investigation, we report the isolation and identification of a total number of 21 blue green algae from Perambalur lake, Tamil Nadu, India. Morphological and molecular characterization of one of the unique isolates, PSL17 aided in authenticating the identity of the cyanobacterium, revealing its close relation to the genus *Desertifilum*. Various growth parameters such as light intensity, pH, temperature and aeration were optimized. Our results indicate that the optimal growth was obtained in pH between 6.8-7.0 and temperature range of 30-31°C under constant light condition with a vigorous swirl once every day. These conditions favored the remarkable growth of *Desertifilum* sp. leading to increased biomass production accentuating the significance of this blue green alga in the field of industrial and biotechnological applications. The 20 other blue green algae identified in the present study are *Anabaena, Aphanothece, Aphanizomenon, Arthrospira, Calothrix, Chroococcidiopsis, Chroococcus, Cylindrospermopsis, Cylindrospermum, Gloeocapsa, Lyngbya, Merismopedia, Microcytis, Nostoc, Oscillatoria, Phormidium, Planktothrix, Rivularia, Spirulina*, and *Synechococcus*. This investigation reports the diversity of the blue green algae in Perambalur lake and gives a primary understanding and the growth kinetics of *Desertifilum* sp. PSL17 to further explore the research approaches on this potential cyanobacterium in ecological and industrial perspectives.

Keywords: Cyanobacteria; Freshwater; *Desertifilum* sp.; Growth Optimization; Tamil Nadu

Introduction

Cyanobacteria are photosynthetic prokaryotic organisms which have the capability to thrive in a varied aquatic and terrestrial environments [27]. Cyanobacteria are considered to be the oldest form of life on earth. Cyanobacteria occur in freshwater, brackish, and marine environments and are sometimes also found on soils and rocks. Cyanobacteria are known prevalent forms in the majority of habitats, and in addition to the diversity in their forms, they are also contributors of various substances viz. enzymes, pigments, and vitamins [25]. Freshwater habitats are well-known for their cyanobacterial diversity and are known to possess significant biotechnological potential but are not efficiently characterized. Cyanobacteria are considered to be the most significant among microorganisms, in the production of important bioactive metabolites such as phenol compounds, alkaloids saponins, tannins, etc., having vast applications in therapeutics and in the fields of agriculture and biotechnology [22]. Hassan., *et al*. (2022) [14] have reported that cyanobacteria are potential sources among natural resources for the discovery of novel bioactive compounds which are part of their metabolic pathway [14]. Wang and Irving (2011) [31] have reported the ability of cyanobacteria to produce major plant growth regulating hormones such as auxin, gibberellins, cytokinin, ethylene and jasmonic acid.

Desertifilum tharanse, a filamentous blue green algae described earlier from the biological crusts of Thar, India [7]. Apart from crusts of Thar, *Desertifilum* have been reported from diverse habitats viz. warm spring, alkaline pool etc. [5,6]. Zhang., *et al*., (2018) [32] have reported that the occurrence of this alga in broad range of habitats could be due to their enormous physiological features which is letting them grow in diverse environmental conditions. *Desertifilum* sp. has been reported to possess diverse applications. Co- cultivation of *Desertifilum* sp. with *Pseudomonas* enhanced the crop productivity [17]. It has been reported as a potential cyanobacterium producing indole acetic acid and ammonia thereby enhancing the growth of green grams [21]. In addition to this, the ability of *Desertifilum* sp. to biocontrol wilt disease caused by *Fusarium*, *oxysporum* was also reported [2]. *Desertifilum* is being explored extensively by various research groups for its pharmaceutical applications such as antibacterial, antioxidant and anticancer activities [13,19,26]. In the present investigation, we focused on the diversity of the blue green algae along with the detailed characterization of the cyanobacterium *Desertifilum* sp. PSL17 isolated from Perambalur lake, Tamil Nadu, India. The previously reported *Desertifilum* sp. and the one reported in the present study differed in morphology and growth parameters, indicating that *Desertifilum* is capable of growing in diverse habitats. The present study will pave way for understanding the ecological roles of cyanobacteria in freshwater lakes and the importance of *Desertifilum* sp. The optimization study determined the optimal growth condition, which will help explore the full potential of this cyanobacterium. This diverse nature of *Desertifilum* indicates that this could be a potential candidate, and further exploration might lead to understanding the functionality of this species.

Materials and Methods

Sample collection

Samples were collected from the freshwater lake in Perambalur, Tamil Nadu (Figure 1). The samples were collected from the epilimnion layer of the lake. A sample volume of 1 L water was collected in sterile containers and brought to the laboratory for the isolation and purification of blue green algae.

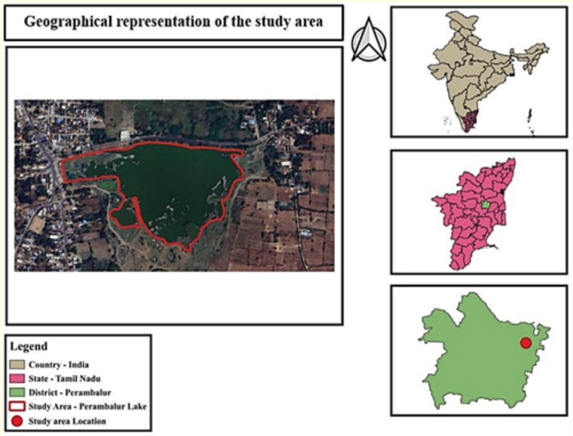

Figure 1: Geographical representation of the study area.

The marked zone is the study area of the present investigation, Perambalur lake of Tamil Nadu, India.

Isolation and purification of cyanobacteria

Isolation of the cyanobacteria species from the lake water sample was carried out by standard methods, viz. serial dilutions, plating, colony isolation, and maintenance of pure culture techniques. BG11 (+N) medium was used throughout the study to grow the blue green algal cultures. Pure cultures of cyanobacteria were isolated by serial dilutions using BG11 medium by the plate culture method [24]. One mL of the diluted samples was transferred to a BG11 plate and evenly spread across the surface, and the algae were allowed to grow for about 7 to 10 days by incubating under continuous light at 30°C. For subculturing, the isolated colonies of the algae were streaked onto BG11 medium. This streaking method was repeated until the exclusion of unialgal and pure axenic cultures was obtained. The isolated cyanobacteria were maintained in BG11 medium and stored in a light and temperature-maintained condition by repeated plating on BG11 medium.

Cultivation of pure cultures of cyanobacteria

The isolated colonies of cyanobacteria were grown in 1 mL of BG11 medium and then transferred to a 4 mL medium, followed by scaling up of medium to 500 mL. The pure cultures were maintained by subculturing in 100 mL of sterile BG11 medium in a 250 ml Erlenmeyer flask. The culture flasks were incubated under continuous light at 30°C giving a vigorous swirl once every day. The pH of the BG11 medium was maintained between 6.0 and 8.0. After the observation of complete growth, which usually takes up to 21 days, the culture was used for further analysis.

Morphological characterization and identification of the cyanobacteria

Cyanobacterial isolates were initially segregated based on the morphology of the colonies on BG11 agar medium, followed by microscopic analysis. The morphology of the cyanobacteria was observed using a light microscope at different magnifications, such as 10x, 40x, and 100x. Critical observations were carried out for all the isolates. Parameters such as shape, size, and color were analyzed, documented, and photographed. Taxonomic identification up to the genus level was carried out using monographs, literatures, and online sources [11]. All the cultures were labelled and maintained for further study. The isolate PSL17 was unique and appeared novel compared to the other isolates, and hence, was taken for further analysis for which, the isolate was cultivated in BG11 medium in a 2000 mL Erlenmeyer flask followed by incubation in continuous light at 30°C. The pure culture was maintained by subculturing in 100 mL of sterile liquid BG11 medium and incubated under continuous light at a temperature of 30°C. The biomass of the culture was harvested by centrifugation and used for further analysis.

SEM analysis of the isolate PSL17

Scanning electron microscopy (SEM) analysis was performed with slight modifications of the protocol given by Dadheech., *et al*., (2014) [6] to visualize the algal filaments. Prior to fixing in 3% glutaraldehyde, the cells were given a wash with 0.1 M phosphate buffer, pH 7.0. The mixture was incubated at room temperature for 4 h followed by washing the cells in 0.1 M phosphate buffer. The cell pellet was resuspended in 2% OsO4 solution, and incubated for 30 min. The cells were dehydrated using increasing concentrations of acetone (successively 30%, 50%, 70%, and twice 100% for 30 min). The samples were dried on silica gel by placing in a desiccator for 24 h. The surface of the algal cells were coated with gold with a sputtering machine followed by analysis using a Zeiss scanning electron microscope.

Extraction of genomic DNA from the isolate PSL17

Among all the isolates, isolate PSL17 was taken further for molecular characterization as this isolate appeared unique. Genomic DNA was extracted from a 50 mL culture and the cell pellet was resuspended with a 400 µL of lysis buffer (Urea 4 M; Tris–HCl 0.2 M, pH 7.4; NaCl 20 mM and EDTA 0.2 M) and 50 µL of Proteinase K (stock solution of 20 mg/mL) and the mixture was pipette mixed immediately. This was followed by incubating the mixture for 1 h at 55°C giving frequent pipette mixing at regular intervals. To this mixture, 1 mL of prewarmed (55°C) DNA extraction buffer made of CTAB 3%; NaCl 1.4 M; EDTA 20 mM; Tris–HCl 0.1 M, pH 8.0; Sarkosyl 1% and Mercaptoethanol 1% solutions were added and the tubes were gently mixed and incubated at 55°C for 1 h giving a frequent mix every 10 min. To the mixture was added double the volumes of chloroform: isoamyl alcohol (24:1 v/v) and was given a gentle mix followed by centrifugation. The upper phase was pipetted out to a fresh tube and was added with double the volumes of absolute ethanol, and stored at -20°C for 1 h followed by centrifugation. The pellet was given a 70% ethanol wash followed by air drying and dissolving the pellet in 50 µL of sterile water [28]. The quantity and quality of the genomic DNA of the isolate PSL17 was assessed using a spectrophotometer at 260 nm and agarose gel electrophoresis respectively.

Amplification and sequencing of 16S rRNA of the isolate PSL17

The 16S rRNA gene was amplified using the genomic DNA as a template. Universal primers, 16S rRNA forward (5'AGAGTTTGATC-

CTGGCTCAG-3') and reverse (5'- GGTTACCTTGTTACGACTT- 3') were used to amplify the 16S rRNA gene and final PCR reaction was adjusted to a final volume of 25 µL [9]. The PCR conditions were for 30 cycles of 94°C for 10 sec, 60°C for 20 sec and 72°C for 60 sec. The PCR product was run on an agarose gel to check for amplification and the PCR product was gel eluted for sequencing. Automated BigDye terminator sequencing reactions were performed using the PCR product and the appropriate primers followed by sequencing.

Sequence analysis, genbank submission, and phylogenetic analysis

The sequence of the 16S rRNA gene was analyzed for sequence similarity and multiple sequence alignment using the NCBI Blast tool and ClustalW, respectively. Following the similarity search, the sequence was submitted in GenBank. After thorough analysis, the phylogenetic tree was constructed for the 16S rRNA gene isolated from PSL17 with ten closely related species using the free version of MEGA11 software [3].

Growth Optimization of *Desertifilum* sp. PSL17

The pure culture was maintained in sterile BG11 medium. The culture conditions for the production of *Desertifilum* sp. PSL17 were optimized by growing the culture at varied pH and temperatures ranging from 6-9, and 24-32°C respectively. The light intensities tried were 12h light and 12h dark cycles and a continuous light exposure. Influence of aeration was also studied by incubating two sets of culture flasks, one under stationary condition and the other was given a vigorous swirl once every day. The culture flasks were incubated till the complete growth was observed which usually attains by day 21. The optimum culture conditions for the production of *Desertifilum* sp. PSL17 were determined.

Results and Discussion

The present study contributes to the fundamental understanding of the blue green algal diversity of freshwater habitats. A wide range of blue green algal isolates were obtained from the water sample of Perambalur lake, Tamil Nadu. The pH of the lake water was between 7-7.3 and the temperature was between 30-32°C. Microscopic analysis aided in understanding the morphology of the isolates followed by the taxonomic identification of the isolates up to genus level using monographs, literatures and online sources [11]. The morphology of the isolates was ranging from circular to filamentous which indicates the richness of habitat. A total number 21 blue green algae were identified from the water sample belonging to various orders of cyanophyceae (Plate 1, Table 1).

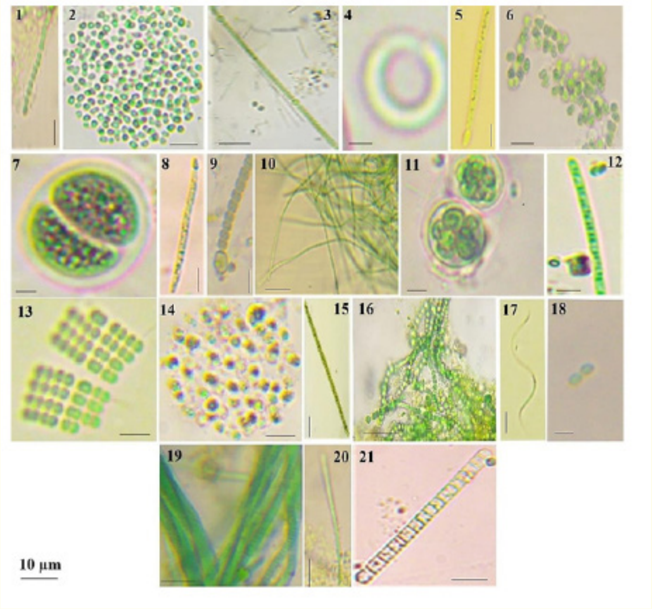

Plate 1: Blue green algae isolated from the freshwater lake from Perambalur, Tamil Nadu.
1. *Anabaena* 2. *Aphanothece* 3. *Aphanizomenon Flos- Aquae* 4. *Arthrospira* 5. *Calothrix* 6. *Chroococcidiopsis* 7. *Chroococcus* 8. *Cylindrospermopsis* 9. *Cylindrospermum* 10. *Desertifilum* 11. *Gloeocapsa* 12. *Lyngbya* 13. *Merismopedia* 14. *Microcystis* 15. *Planktothrix* 16. *Nostoc* 17. *Spirulina* 18. *Synechococcus* 19. *Oscillatoria* 20. *Rivularia* 21. *Phormidium*.

S. No.	Genus	Order	Family
1	*Anabaena*	Nostocales	Aphanizomenonaceae
2	*Aphanothece*	Chroococcales	Microcystaceae
3	*Aphanizomenon Flos- Aquae*	Nostocales	Aphanizomenonaceae
4	*Arthrospira*	Oscillatoriales	Microcoleaceae
5	*Calothrix*	Nostocales	Rivulariaceae
6	*Chroococcidiopsis*	Chroococcidiopsidales	Chroococcidiopsidaceae
7	*Chroococcus*	Chroococcales	Chroococcaceae
8	*Cylindrospermopsis*	Nostocales	Aphanizomenonaceae
9	*Cylindrospermum*	Nostocales	Aphanizomenonaceae
10	*Desertifilum sp*	Desertifilales	Desertifilaceae
11	*Gloeocapsa*	Chroococcidiopsidales	Aliterellaceae
12	*Lyngbya*	Oscillatoriales	Microcoleaceae
13	*Merismopedia*	Chroococcales	Microcystaceae
14	*Microcystis*	Chroococcales	Microcystaceae
15	*Nostoc*	Nostocales	Nostocaceae
16	*Oscillatoria*	Oscillatoriales	Oscillatoriaceae
17	*Phormidium*	Oscillatoriales	Oscillatoriaceae
18	*Planktothrix*	Oscillatoriales	Microcoleaceae
19	*Rivularia*	Nostocales	Rivulariaceae
20	*Spirulina*	Spirulinales	Spirulinaceae
21	*Synechococcus*	Synechococcales	Synechococcaceae

Table 1: Blue green algae isolated from the freshwater lake from Perambalur, Tamil Nadu.

The phytoplankton in the higher trophic levels of most of the lakes are dominated by cyanobacteria viz. *Anabaena*, or *Aphanizomenon*, *Limnothrix*, *Microcystis*, and *Planktothrix*. This persistent cyanobacterial ascendancy is, therefore, regarded as the conclusive phase of eutrophication occurring worldwide [8]. Valerio., *et al*. (2009) [30] have reported the dominance of species belonging to the order chroococcales, oscillatoriales and nostacales in freshwater reservoirs. In the similar manner, in the present study, we report the wide range of isolates belonging to the orders nostocales and oscillatoriales followed by chroococcales. There were two members belonging to the order chroococcidiopsidales one belonging to spirulinales and synechococcales each. Though, a few of the members belonging to some of these orders is known for their toxic activity, some are known for their applications in agriculture, medicine and biotechnology industries [1].

Among all the 21 isolates, one isolate PSL17 appeared intriguing and distinct and hence was taken for further analysis. Detailed light microscopic and SEM analysis revealed the characteristics such as cell structure, form and the extent of constriction between adjacent cells. Light microscopic analysis revealed the isolate to be filamentous in nature being light green in color. The filaments were thin and the cells were elongated and rectangular in nature and were arranged in a uniseriate pattern and the filaments were in varied length and were densely entangled. Though, this isolate was sharing features of members belonging to oscillatoriales [16], certain characteristic features of this isolate was unique and thus preparation of mass production and characterization was carried out to identify the isolate. The cells were rectangular in shape with length more than the width of the cell and the apical cell was cylindrical in shape. The width of the cell was between 2 to 2.5 μm and a length of cell was between 4-6 μm (Figure 2). There was constriction at the cross walls of the filaments and the filaments were lacking heterocyst and akinetes.

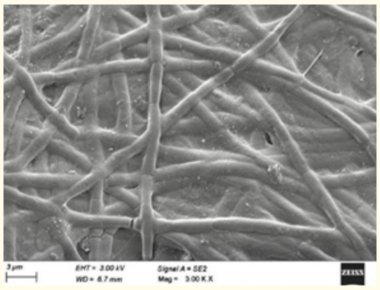

Figure 2: SEM image of *Desertifilum* sp. PSL17.

The SEM analysis revealed the filamentous nature of the alga exhibiting constriction at the cross walls of the filaments.

Microscopic analysis was not sufficient to identify the isolate PSL17 and hence molecular characterization was performed for the authentication of the isolate. The 16S rRNA gene of 850 bp sequence when performed for sequence similarity in NCBI Blast tool, revealed that the 16S rRNA sequence was sharing 99% homology with the 16S rRNA sequence of *Desertifilum* sp. which was available in the GenBank database with accession number KU556389 [4]. Phylogenetic tree constructed based on the analysis of 16S rRNA conducted in MEGA11 software revealed that the isolates clustered together to the member of well characterized and previously named species, affiliated to the order – oscillatoriales. Neighbour-joining tree revealed the phylogenetic position of this cyanobacterium to be closely related to the previously reported *Desertifilum* sp. (Figure 3). The 16S rRNA sequence was also sharing homology with the 16S rRNA sequence of *Desertifilum tharanse*, *D. fontinales*, and *D. salkalinema* [5-7]. The phylogenetic tree indicated that *Desertifilum* belongs to the class (Cyanophyceae), order (Desertifilales), family (Desertifilaceae). The sequence was submitted in the GenBank with the accession number OP035057 with the name *Desertifilum* sp. PSL17 [20]. The present study revealed this blue green alga differ in morphology and the growth parameters indicating that *Desertifilum* is capable of growing in diverse habitats. This finding suggests that this alga could have a wide ecological adaptability than perceived. Recently, this species is being studied by various groups for its pharmaceutical applications such as antioxidant and anticancer activities [13,26].

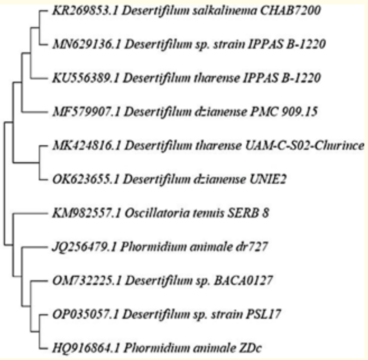

Figure 3: Phylogenetic tree of 16S rRNA of *Desertifilum* sp. PSL17 and related genus.

Phylogenetic tree of 16S rRNA of *Desertifilum* sp. PSL17 illustrating the evolutionary relationship of this genus and the related genus.

Different parameters such as light, pH, aeration and temperature were used to optimize the growth of *Desertifilum* sp. PSL17. The important growth parameters like light and temperature have a direct impact on the community dynamics of the blue green algae. Some of the cyanobacterial members were known to exhibit luxurious growth between 20-40°C. When varied temperatures such as 24 to 32°C were used, it was found that the isolate was growing well in the temperatures ranging between 30-31°C and similarly when the culture was grown in pH ranging between 6 to 9, it was revealed the growth of the isolate was remarkable in pH between 6.8-7.0 Previous study has shown that peak growth was observed when cyanobacteria was grown in the temperature range 25-30°C [23], which is well aligning with our present study. The pH on growth optimization of *Spirulina platensis* has favored the overproduction of antioxidants and chlorophyll content [15]. Light is an important parameter and the isolate when grown in presence of continuous light was found to be better than growing the culture under 12h light and 12h dark cycle. This observation was in accordance with the previous reports on cyanobacteria, which demonstrates the crucial role of light intensity in regulating photosynthetic activity and the production of biomass [10]. The culture medium composition for improving growth and lipid content of green microalgae *Chlorella sorokiniana*, *Scenedesmus acuminatus* and blue green *Cyanobacterium aponicum* led to the elevated production of lipid, a possible biomaterial for biodiesel production [12]. Reshma., *et al*. [18] reported the enhancement of pigment production in *Dunaliella salina* under optimized conditions and this alga is considered to be a prospective feedstock for pigments. In addition to the aforesaid parameters, aeration was also studied and it was revealed that the isolate grew well in aerated culture than the still culture. *Chlorella* spp. is known for the production of exopolysaccharides (EPS) and it has been reported that optimizing the growth conditions resulted in the enhancement of EPS production, making it a promising candidate for industrial applications [29]. The present investigation helped in identifying important environmental factors that significantly affect the growth and production of biomass of *Desertifilum*. Our earlier investigations on this alga on the induction of rice germination and growth promotion in green gram and the antibacterial activity indicated the potential of this alga in the field of agriculture and medicine [19,21]. The isolation and identification of the cyanobacterium *Desertifilum* sp. PSL17 from the Perambalur lake, Tamil Nadu, India contributes to the growing wisdom of this cyanobacterium as they have been previously discovered from extreme environments. Investigation of growth parameters have given inclusive knowledge of the ecological adaptability of *Desertifilum* sp. PSL17, which reveals that this species could be a potential candidate for many biotechnological and industrial applications.

Conclusion

The isolation and identification of cyanobacterial species of water samples collected from Perambalur lake of Tamil Nadu led to the isolation of 21 cyanobacterial species. Most of the isolates were belonging to the order nostocales and oscillatoriales. Two isolates were belonging to chroococcales and chroococcidiopsidales and one belonging to spirulinales and synechococcales each. A unique filamentous algae PSL17, resembling the members of oscillatoriales was of our interest and the detailed microscopic analysis revealed the isolate to be filamentous with filaments of varied lengths and densely entangled. The cells were elongated, cylindrical with the apical cell being short and dome shaped with no heterocyst and akinetes. SEM analysis revealed the size, shape and constriction at cell junctions. Molecular characterization of the isolate PSL17 revealed the identity of the alga as *Desertifilum* and hence designated as *Desertifilum* sp. PSL17. *Desertifilum sp. PSL17*. exhibited a distinct constriction in the cross wall whereas only moderate constriction was observed in the *Desertifilum*. *Desertifilum* species have been isolated from diverse and intense habitats such as Thar crust, hot spring and alkaline lake but interestingly our study is the first report of this alga being isolated from freshwater of Perambalur lake. This adaptive nature of *Desertifilum* sp. and its ability to withstand extreme conditions can make this a potential candidate. Our earlier studies on the plant growth promoting ability and the antibacterial activities of this alga were promising and implies the prospects of this alga in the field of agriculture and medicine respectively. The present investigation was successful in identifying and optimizing the growth parameters for *Desertifilum* isolated from Perambalur lake, Tamil Nadu, India indicating its significance for industrial applications. Thus, further exploration of the novel *Desertifilum* sp. obtained in the present study might pave way for the discovery of important compounds with significance in the field of agriculture, cosmetics, food, biotechnology and pharmaceutical industries.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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