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Biosurfactant and siderophore producing endophytic fungi from duckweed

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Abstract

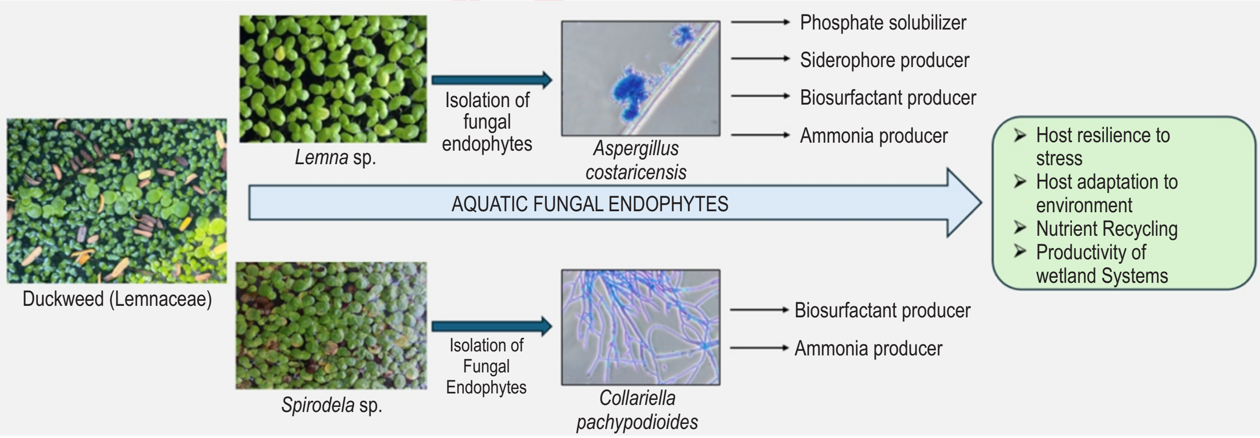
Aim: Fungal endophytes are vital for climate resilience and adaptability of host plant. Although terrestrial plant associated endophytes are well studied, aquatic endophytic fungal diversity is still under explored. The aim of this paper was to isolate the fungal endophytes from floating aquatic plants and assess their ecological significance.

Methodology: Endophytic fungi from two different duckweed species, *Lemna* and *Spirodela,* were isolated and identified. Functional attributes, including production of ammonia, indole-3-acetic acid (IAA), and siderophores and phosphate solubilization were estimated.

Results: Two endophytic fungi, *Aspergillus costaricensis* and *Collariella pachypodioides* (*Chaetomium pachypodioides*), were isolated from *Lemna* sp. and *Spirodela* sp. *Aspergillus costaricensis,* reported for the first time as an endophyte in the aquatic plant *Lemna*, was able to solubilize phosphate and produce ammonia and siderophore. Both the strains were biosurfactant producers, an attribute not previously documented for aquatic plant endophytes.

Interpretation: Both the fungal isolates appeared to play an active role in nutrient recycling in the host environment. Duckweed plants are vital for the functioning of wetland ecosystems. Unexplored fungal species may have unique biochemical pathways critical for degrading pollutants or enhancing nutrient availability in wetlands, attributes that will be useful for establishing nature based solutions and productive aquatic farming systems.

Key words: *Aspergillus,* Biosurfactant, Duckweed, Endophytes, Wetlands



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Introduction

Plant associated endophytes have emerged as an ecologically and physiologically important class of bacteria and fungi that impart beneficial advantages to the host plant, including protection against biological and abiotic stresses, production of bioactive and biocontrol molecules and enhancement in plant growth (Chitnis *et al.,* 2020; Rai *et al.,* 2021). Endophytic microbial diversity and abundance are influenced by multiple factors, including host plant species, plant's growth stage, environmental conditions and geographic location (Gehring *et al.,* 2017). Hence, the plant microbiome of each plant is structurally and functionally diverse and is a potential source of novel fungal and bacterial flora with varied functions pertaining to the host plant or surrounding environment. Although the role and significance of endophytic bacteria is well established for all plant forms, studies on the plant endophytic fungi, in comparison to bacterial endophytes, are less (AlKahtani *et al.,* 2020; Verma *et al.,* 2022). Further, reports on the taxonomic and functional diversity of fungal microflora of aquatic plants are limited (Almeida *et al.,* 2015; Govindan *et al.,* 2020).

Aquatic fungi contribute to ecosystem functioning through nutrient cycling, organic matter decomposition, and by establishing beneficial associations that enhance plant growth and resilience. These interactions support the health and productivity of aquatic ecosystems, which are critical for maintaining water quality, biodiversity, and ecological balance (Keller *et al.,* 2018; AbuQamar *et al.,* 2023). Hence, the study of fungal endophytes from different aquatic plants represents a novel frontier in the context of Environmental, Social and Governance (ESG) framework, offering promising implications for environmental sustainability, pollution control, biodiversity conservation, community engagement and resource recovery (Tiwari and Park, 2024). As aquatic environments face increasing stress from anthropogenic activities and climate change, studying these fungal communities offer insights into nature-based solutions that can promote sustainable ecosystem management and facilitate the development of environment friendly technologies. Exploration of underexplored fungal habitats and functionalities can lead to the discovery of novel fungal candidates as eco-friendly bioinoculants, useful for sustainable aquaculture, community wastewater treatment and environmental stewardship. These will go a long way in ensuring resilience, functionality and productivity of wetlands and other aquatic systems.

Lemnaceae (commonly called duckweed) are the smallest flowering freshwater plants, widely studied for their water remediating properties (Singh *et al.,* 2023) and numerous health benefits (Appenroth *et al.,* 2016). Like other plants, microbiome of duckweed has been found to play a determining role in the functional performance of the plant (Jewell *et al.,* 2023). There are reports on the colonization of *Lemna* and *Spirodela* by diverse endophytic bacteria and yeast and their role in plant growth promotion and stress response evolution is well established (Kajadpai *et al.,* 2023; Pramanic *et al.,* 2023). Duckweed microflora has been a source of many novel bacteria (Saimee *et al.,* 2024). There are limited studies on the fungal microflora associated with duckweed. *Pythium myriotylum* as a phytopathogen was reported from *Lemna gibba* (Flaishman *et al.,* 1997). In another work, *Tracya lemnae* was identified as a phytopathogen for *Spirodela polyrhiza* (Van Steenwinkel *et al.,* 2022). However, the taxonomic and functional studies on the fungal endophytes from duckweed are lacking.

In view of the above, this study was undertaken to isolate and functionally characterize fungi from two different duckweed *Lemna* and *Spirodela,* cultivated on domestic wastewater. The novelty of this work lies in the exclusive study of fungal endophytes associated with duckweed and their role in the functional attributes of host plant species. Endophytic fungi associated with duckweeds and other wetland flora may possess unique metabolic traits that enhance the plant's ability to tolerate pollutants, degrade contaminants, or sequester nutrients, thereby contributing to cleaner water bodies, healthier ecosystems, and higher climate resilience.

Materials and Methods

Collection of duckweeds: Duckweeds were collected from the Mula river, at Aundh, Pune, India and Zonal Agricultural Research Station, Pune, India. Collection was done in the month of February (temperature 30 °C). The plants were identified on the basis of morphological characteristics as *Lemna* sp. and *Spirodela* sp. (Fig. 1). The identification of duckweeds was confirmed as per details provided in the previous reports (Appenroth *et al.,* 2016) and validated by Dr. M. Dhanorkar, botanist, Symbiosis International (Deemed University), Pune, India. These plant samples were segregated and maintained in separate fabricated metal tank at the Symbiosis International (Deemed University), Lavale, Pune, India in untreated domestic sewage water for further growth and development of monoculture (Zuki *et al.,* 2022). Day temperature during the study period varied from minimum 25 °C to maximum 35 °C, while night temperature varied from minimum 15 °C to maximum 25 °C. The average day temperature during the period of study was 30 °C. The containers were placed in open in an area receiving diffused sunlight. Plants were allowed to grow for a minimum of four weeks in untreated domestic sewage water before harvesting for further studies.

Isolation of fungal endophytes: Healthy plantlets (approximately 25-30) were collected from multiple points from the in house-maintained untreated domestic sewage water tank. The collected plant samples were pooled together and divided in three sets. After thorough washing with tap water, the plants were washed with sterile distilled water under aseptic conditions, followed by 70% ethanol and finally by 1% sodium hypochlorite, each treatment lasting for 5-7 min. (Ezra *et al.*, 2003). Post sterilization, all the plants were rinsed multiple times with sterile distilled water under aseptic conditions. Few plants were placed individually on nutrient agar (NA) and potato dextrose agar (PDA) surface to confirm surface sterility. One set of plants was treated

Fig. 1: Duckweeds in the current study. *Lemna* sp. contained small leaves (3-4 mm) and single root per thallus. Whereas the *Spirodela* sp. was characterized by larger leaves (7-8 mm), reddish underside and the more extensive root system.

with 1% sodium hypochlorite for a longer period of time (10 min) and placed on sterile NA and PDA media. Water obtained after the final water wash was also plated on NA and PDA media to reconfirm the sterility of treated plant specimens. Sterile plants were crushed, and a suspension was prepared in sterile normal saline (0.9%). Aliquots from the suspension were plated, in triplicates, on PDA supplemented with Rose Bengal and incubated at 30 °C for 5-6 days. The fungal isolates obtained were sub-cultured onto fresh PDA plates and maintained at 4 °C for further studies. Small cubes of fungus, grown on PDA medium were cut, inoculated in 20% glycerol solution and stored at -20 ^{0}C for future use (Hwang, 1960; Homolka *et al.*, 2003).

Identification of the fungal isolates: The fungal isolates were identified using morphological features and ITS region sequencing. For the identification of fungi, the Internal Transcribed Spacer (ITS) region of the 18S rRNA gene was sequenced. CTAB method was used to extract the genomic DNA from pure cultures (Umesha *et al.,* 2016). DNA in samples was confirmed and quantified using a NanoDrop Microvolume Spectrophotometer (Thermo Fisher Scientific). ITS region was amplified using universal fungal primer ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4(5'TCCTCCGCTTA TTGATATGC-3'). PCR amplified product was purified using PEG-NaCl (White *et al.*, 1990) and further sequenced using ABI Sequences and BigDye Terminator Chemistry Applied Biosystem as per Sanger's method (Sanger *et al.*, 1977). Contigs were generated from the raw data using SeqMan, and a similarity search was conducted using the NCBI database (Altschul *et al.,* 1997). The gene sequences of two isolates were submitted to the GenBank database with accession number PP422969 and PP422970 for STWF1 and LTWF1 respectively. These are available at the following URL: https://www.ncbi.nlm.nih.gov/nuccore /PP422969.1/ for STWF1 and https://www.ncbi.nlm.nih.gov/nuccore/PP422970 for LTWF1.

Screening of isolates for functional traits: Isolated fungal isolates were assessed for plant growth promoting traits.

Ammonia production: Fungal isolates were inoculated in 5 ml of 1% Proteose peptone broth and incubated at 30 °C for 24-48 hr at 150 rpm in an orbital shaker. The culture suspension was centrifuged at 8000 g for 15 min to remove the biomass. Nessler's reagent was added to the cell free supernatant and observed for the development of orange to brown color indicating production of ammonia by the test organism. The appearance of faint yellow colour indicated low concentration of ammonia, while a deep yellow to brownish colour signified the maximum ammonia production. Sterile medium, without any inoculation, was taken as a negative control (Cappuccino and Sherman, 1992).

Indole-3-acetic acid (IAA) production: Fungal isolates were inoculated in a potato dextrose broth amended with L- tryptophan and incubated for 48-72 hr at 30 °C. Salkowski reagent was added to the culture supernatant and observed for colour change after 20 min of incubation at room temperature in dark. Development of dark pink / red colour indicated the presence of indole acetic acid (Patten and Glick, 2002). Sterile uninoculated media served as a negative control.

Phosphate solubilization: Fungal isolates were inoculated on Pikovskaya's medium and inoculated at 30 ^{0}C for 5-6 days (Jasim *et al.,* 2013). Formation of a clear zone around the fungal colony after 5-6 days of incubation suggested the ability of the isolate to solubilize phosphate. Phosphate Solubilization Index (PSI) was calculated by measuring the clear zone around the colony as described in Pikovskaya (1948); Edi-Premono Moawad and Vleck (1996).

Siderophore production: Production of siderophore was estimated by the chrome azure S (CAS) agar method adopted from Louden *et al.* (2011). CAS dye reagent (solution 1) was added to $FeCl_3.6H_2O$ solution (solution 2) and the resultant solution was slowly mixed with cetyltrimethylammonium bromide (CTAB) solution (solution 3) to give the blue dye. Casamino acid solution, 1,4-piperazinediethanesulfonic acid (PIPES), glucose (20%) and the blue dye were mixed with sterile minimal medium9 containing agar, and poured in plates to make medium plates. Fungal isolates were inoculated and incubated for 4-5 days at 30 °C. Indication of siderophore production was confirmed by the development of orange-red coloration around the fungal colony.

Biosurfactant production: Fungal isolates were grown in proteose peptone glucose acid salt (PPGAS) medium (pH 7.2) containing NH_4Cl (1M), KCl (1M), Tris-HCl (10M), $MgSO_4$ (1M), glucose (10%), and 20% peptone. Screening for biosurfactant production was done on the cell free culture broth using three different assays: Drop collapse assay, oil spread technique and emulsification assay, as detailed in Singh *et al.* (2021).

Chemicals, Reagents, and Media: Microbiological media, including Pikovskaya's agar, nutrient broth, potato dextrose broth, and proteose peptone broth, were procured from Hi Media Pvt. Ltd., Mumbai, India. All the other chemicals used were of analytical grade.

Statistical analysis: All the above tests were performed in triplicate. One-way ANOVA was used to evaluate the significant difference in phosphate solubilization. GraphPad PRISM software was used for the analysis.

Results and Discussion

Taxonomically and functionally diverse bacterial species have been previously isolated from the duckweed endophytic microflora (Saimee *et al.,* 2024). However, to the best of our knowledge, isolation and functional studies on the fungal flora from duckweeds is lacking. Duckweeds samples, collected from natural water bodies were found to be a polyculture consisting of *Lemna* and *Spirodela* sp. The plants were identified morphologically, with the prophyllum of *Spirodela* sp. clearly identified and absence of prophyllum in *Lemna* sp. The former genera can be instantly identified by its slightly larger size, reddish undersides and more extensive root system than the latter genera (Bog *et al*., 2020). Hence, these plants were successfully segregated and grown in separate containers for further study. Untreated domestic wastewater, with nutrient content suited for duckweed growth was used for maintaining the plants (Singh *et al*., 2023).

Duckweeds have phytoremediation properties, hence the use of wastewater ensured the use of low-cost medium for duckweed growth. Use of wastewater also mimicked the natural conditions that support the proliferation of duckweed, especially in wetland ecosystems, making the study sustainable. Isolation of endophytes was done after complete surface sterilization of the selected plants. No microbial growth was observed on the plants when placed on solid medium post sterilization, confirming the sterility of plant surface. Microbial growth was observed only when the plants were crushed, enabling the microflora from inner tissues to be exposed. Additionally, water from the last washing of the plant also showed no microbial growth on media plates confirming the sterile surface of the plants under study. These results confirmed successful surface sterilization of plants, establishing the endophytic nature of fungal isolates obtained.

One fungal endophyte from Spirodela (STWF1) and one isolate from *Lemna* (LTWF1) were obtained on PDA medium (Fig. 2). The isolate STWF1 was found to be a fast-growing fungus, forming yellow coloured colony with reverse hyaline. No fruiting bodies were produced even after 15 days of incubation. LTWF1 colony was compact and slow growing, with hyaline and fluffy mycelium. Colony was reverse, hyaline was present and colony centre was raised with dark blackish-brown conidial heads. Sporulation was abundant and heads were globose to radiate and dark brown in colour. Microscopic observation of STWF1 revealed vegetative hyphae, single celled conidia, globose, geniculate conidiophores, oblong to cylindrical, truncated with prominent scar at one or more ends, smooth walled and pigmented. Conidiophores walls of LTWF1 were smooth, sterigmata was biseriate and conidia globose to elliptical and echinulate (Fig. 3).

NCBI BLAST results revealed more than 99% homology of isolate STWF1 with *Collariella pachypodioides*. Percentage similarity between the isolate found in this study and reference strain CBS 164.52 was 99.47%. Isolate LTWF1 showed more

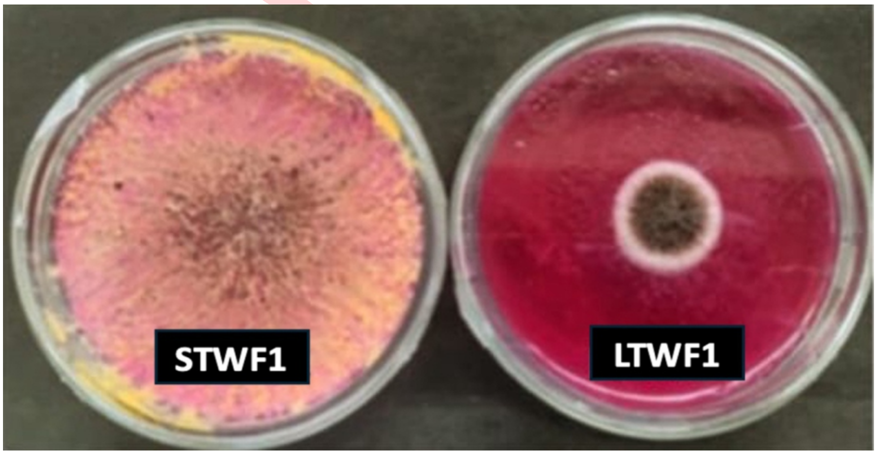

Fig. 2: Colony morphology of fungal isolates from duckweeds when grown on potato dextrose agar medium. (L) STWF1 isolated from *Spirodela* sp. and (R) LTWF1 isolated from *Lemna* sp.

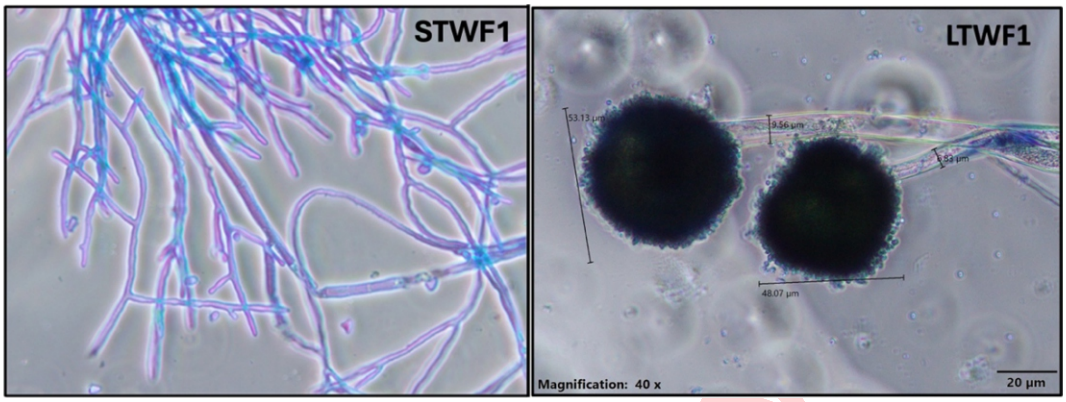

Fig. 3: Microscopic examination of fungal isolate STWF1 from *Spirodela* and LTWF1 from *Lemna* sp.. STWF1 was characterized by absence of fruiting body and globose single celled conidia. Conidiophore walls of LTWF1 were smooth, with biseriate sterigmata.

than 99% homology with *Aspergillus costaricensis* strain CBS 115574. Based on the morphological characteristics and ITS analysis, the isolates STWF1 and LTWF1 were confirmed to be *Collariella pachypodioides* and *Aspergillus costaricensis* respectively. *Collariella pachypodioides* was earlier known as *Chaetomium pachypodioides* (Wang *et al.,* 2022). *Chaetomium pachypodioides* has been previously reported from a garden soil in Andhra Pradesh, India (Rao *et al.,* 1963). However, to date, there is lack of research on the functional attributes of these fungi in plant life. *A. costaricensis* is a member of the Nigri section of the widely explored black *Aspergilli,* containing other species such as *A. niger, A. tubingensis, A. foetidus, A. piperis, A. brasiliensis, A. ibericus, A. uvarum, A. vadensis* and *A. lacticoffeatus.* This endophyte was first isolated in the rhizosphere at Gaugin Garden on Taboga Island, Costa Rica, producing large pink to greyish brown sclerotia (Samson *et al.,* 2004). It was reported to be negative for ochratoxin production, a trait that could be advantageous for use of *A. costaricensis* for various biotechnological applications. Till date there is no report on any kind of toxicity by *A. costaricensis,* and hence the application of this isolate for generating useful secondary metabolites and in agriculture is promising (Lei *et al.,* 2022). Limited reports on successful characterization of biosynthetic pathways of secondary metabolite are available for *Aspergillus* sp. section Nigri (Wang *et al.,* 2023). Studies like the current work, reiterate the importance of exploring the functional attributes of *A. costaricensis* from diverse habitats, including aquatic plants growing under different environmental conditions.

Although *Aspergillus* and *Chaetomium* sp. have been earlier reported from *Pistia,* their species level identification remains unknown (Kongjornrak *et al.,* 2019). Further, there are no documented reports on the distribution and functional attributes of *A. costaricensis* or *Collariella pachypodioides* (*Chaetomium pachypodioides*) as endophytes from aquatic plants. This is the first report on the isolation of *A. costaricensis* as endophyte from *Lemna* and *Collariella pachypodioides* (*Chaetomium pachypodioides*) as endophyte from *Spirodela* sp. The fungal endophytes isolated in the current study were assessed for multiple plant growth promoting traits, production of IAA, siderophores, biosurfactant, ammonia and phosphate solubilization. Many microorganisms, including bacterial and fungal endophytes, produce phytohormone IAA as a catabolic product of L-tryptophan, critical for the diurnal development as well as plant stress response (Gang *et al.,* 2019). The negative nature of both fungal isolates for IAA production, indicates absence of, or lesser involvement of these endophytes in plant growth via IAA production.

Isolate LTWF1 (*Aspergillus costaricensis*) changed the color of the surrounding medium in CAS agar plate to orange-red, and hence, confirmed to be positive for siderophore production (Fig. 4). CAS assay used for screening of siderophore producing

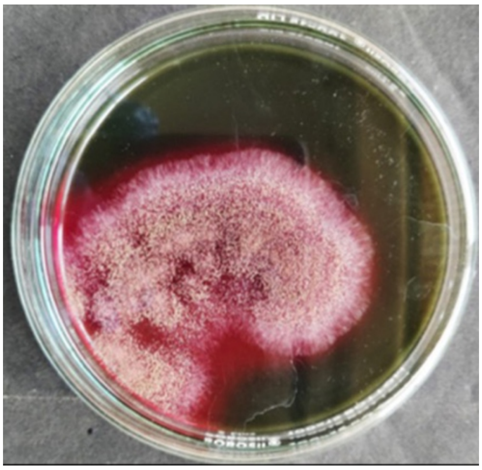

Fig. 4: Positive siderophore production by fungal isolate LTWF1 (*Aspergillus costaricensis*) as evident by the colour change in the CAS agar media (CAS: chrome azure S). Change in colour of the medium to orange red confirmed the siderophore positive nature of LTWF1.

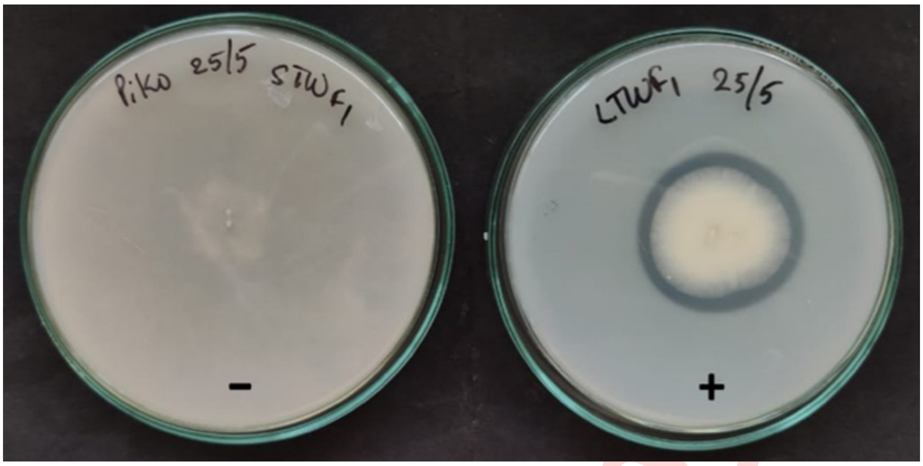

Fig. 5: Phosphate solubilization assay of isolate STWF1 (*Collariella pachypodioides*) and isolate LTWF1 (*Aspergillus costaricensis*). (– indicates negative phosphate solubilization by STWF1, while + indicates positive phosphate solubilizing ability of LTWF1 as indicated by a clearing zone).

ability is based on the formation of a ferric-siderophore complex in the CAS media (Louden *et al.,* 2011). Siderophore produced by the microorganisms break the complex and leads to change in the colour of the medium from blue-green to orange-red, as seen in case of LTWF1 indicating siderophore production by this isolate (Fig. 4). Recent studies have reported the isolation of siderophore producing bacterial endophytes from *Lemna* (Makino *et al.,* 2022). However, there are no reports of siderophore producing fungi associated as endophytes from Lemnaceae. Siderophores have been found to be key determinants in the plant growth and stress response processes, as well as in mitigating the growth of pathogens by way of alteration in iron availability.

Siderophores from plant associated microflora have been found to be actively involved in plant response to heavy metals in the environment, and are key players in the process of phytoremediation of heavy metals from soil, and in alleviating metal toxicity in plants (Syed *et al.,* 2023). Floating aquatic plants are known to be excellent phytoremediators of water bodies and have been used to treat water contaminated with heavy metals (Pang *et al.,* 2023). With elevating pollution levels in water bodies by heavy metals from industrial and domestic sources, it is important to identify the key role played by siderophore producing endophytes from floating aquatic plants in metal remediation and plant metal stress tolerance. Identification and use of siderophore producing endophytes as inoculants in wetlands and other phytoremediation setups would greatly enhance the output from such systems, greatly contributing to ESG framework. However, extensive studies on this aspect are lacking for many aquatic plants.

Fungal isolate STWF1 (*Collariella pachypodioides*) was negative for phosphate solubilization but isolate LTWF1 (*Aspergillus costaricensis*) was found to solubilize phosphate with a phosphate solubilization index of 2.2 (Fig. 5). Phosphate solubilizing test is dependent on the ability of microorganisms to solubilize calcium phosphate present in the Pikovskaya's agar medium resulting in a clear zone around the microbial colony (Fig. 3). The phosphate solubilization index (SI) calculated for the microbial isolates stands as a measure for the extent of solubilization ability of the microorganism. There are reports of isolation of *Acidobacteria* and other bacteria from duckweeds have shown to solubilize phosphate (Makino *et al.,* 2022). However, to the best of our knowledge, there are no reports available on the phosphate solubilizing fungal isolate from *Lemna* or *Spirodela*. Phosphorous is a key limiting nutrient in both terrestrial and aquatic ecosystems. Phosphate solubilizing bacteria play a vital role in phosphate recycling in freshwater systems and marine aquifers (Maitra *et al.,* 2015). Wetlands are a vital ecosystem providing a range of ecosystem services and playing a critical role in climate change mitigation (Teng *et al.,* 2018). Phosphorous transformation and transport in wetland are complex processes, driven by numerous interactions between plant and plant associated microorganisms. Secretion of organic acids or enzymes are considered possible mechanisms by which plant associated microorganisms solubilize phosphate and eventually enhance plant growth (Hassan, 2017). However, exploring the processes of phosphate solubilization in duckweeds mediated aquatic systems by endophytic fungi remains to be explored.

Both the fungal isolates, STWF1 (*Collariella pachypodioides*) and LTWF1 (*Aspergillus costaricensis*), were positive for ammonia production. Production of ammonia by endophytes is an indirect mechanism by which microbial endophytes promote plant growth. Ammonia is primarily used in the formation of different nitrogen containing biomolecules by the plant associated endophytes, thereby enabling the plant to meet their demand of nitrogen and also possibly assist in plant defence against pathogens (Hassan, 2017). However, no earlier reports are available on the production of ammonia by endophytes

Table 1: Functional characteristics of fungal isolate STWF1 (*Collariella pachypodioides*) from *Spirodela* sp. and LTWF1 (*Aspergillus costaricensis*) from *Lemna* sp. (IAA: Indole-3-acetic acid)

Sr. No.	Ammonia production	IAA production	Siderophore production	Biosurfactant production	Phosphate solubilization
STWF1	+	-	-	+	-
LTWF1	+	-	+	+	+

associated with duckweeds. One possible reason for the formation of ammonia by the fungal endophyte can be the breakdown of various macromolecules present in water (Abdel-Hamid *et al.,* 2021). Ammonia has emerged as a global candidate in energy sector, with microalgae being identified as an alternative source to produce green ammonia (Chai *et al.,* 2021). Harnessing the ammonia producing ability of endophytes could open an untapped source of green ammonia. Additionally, this attribute of endophytes could also be of significance when cultivating duckweeds with high protein content for food production purposes (Petersen *et al.,* 2021).

Isolates STWF1 and LTWF1 showed around 50% emulsification, respectively, indicating their ability to produce biosurfactant. Both the isolates were also positive for drop collapse and zone clearing assay, confirming their biosurfactant producing ability. Biosurfactant are structurally and functionally diverse secondary metabolites produced by many microorganisms that play a pivotal role in plant life cycle (Singh and Rale, 2022). They have multiple functions including biocontrol activities, antimicrobial properties and quorum sensing attributes, thereby aiding in plant-microbe communications and pathogen resistance. Table 1 gives an overview of the functional attributes of two fungal isolates from *Lemna* and *Spirodela* (Table 1). Both the fungal isolates in the current study were positive for biosurfactant production indicating their possible role in biocontrol and host defense (Table 1). Considering the attributes exhibited by two fungal isolates, the most probable role of these endophytes is believed to be in the host plant resilience and adaptability to the environment, primarily in nutrient stress.

The current work can act as a basis for more detailed exploration of aquatic fungal endophytes and their active role in aquatic ecosystem dynamics and ecosystem services under the ESG framework. The exact role of the functional attributes explored in the current study in host microbe interactions in these and other aquatic plants under dynamic environmental conditions will be an interesting line of study for future research. It will be interesting to know the role played by *A. costaricensis* in adaptations exhibited by duckweed-cultures, and in ecosystem services provided by wetland and aquatic farming. More extensive isolation regime using different growth medium can be employed to broaden the isolation of endophytes from duckweeds, to arrive at more diverse taxonomic diversity.

The work can be extended to community level taxonomic and physiological profiling using metagenomic data analysis, enzymatic pathway reconstruction and study of extracellular enzyme activity of the microbiome of aquatic plants to decipher the wider biological interactions at play. Since endophytes meet their nutritional requirements from the host plant, study of the shift in fungal population with changing water nutrient levels in situ will be a promising area of research, vital in the ESG framework and climate change resilience of wetland systems.

This study establishes the determinant role of the host plant and surrounding environment on the functional diversity of fungi inhabiting the cells of different duckweeds, and the active role of fungi in host plant nutrient recycling. Understanding the roles of fungi in duckweed ecosystems is crucial for optimizing duckweed cultivation and utilization for multiple uses. The functional role of these fungi as endophytes in aquatic plants, under dynamic environmental conditions, however, needs more exploration. Similar studies across multiple seasons will further aid in understanding the dynamics of fungal endophytes in aquatic plants. Recognizing and managing the dynamics of fungal endophytes will aid in developing sustainable practices for duckweed cultivation, especially in systems like wastewater treatment, wetland systems and as a protein-rich feed source This work is closely aligned with ESG principles. Understanding the role of aquatic fungi in nutrient recycling will aid in strategies for detoxification of polluted environments, improving environmental health, biodiversity conservation in wetland systems, carbon cycling, nutrient recycling, and successful community-level aquatic farming, especially in resource-limited settings—key goals under environmental sustainability. Additionally, symbiotic association between the plant and fungus may make wetlands more resilient to climate change disturbances, thereby making the system vital for sustainable environmental health management.

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Authors' contribution: P. Singh: Visualization, conceptualization, data curation, validation, writing-revision and editing; **K. Yadav:** Fungal identification, software use, data curation; **A. Pramanic:**

Experimentation, data collection, Writing-Original draft; **M. Dhanorkar:** Supervision, project administration.

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Conflict of interest: The authors declare that they have no competing interests.

Data availability: The gene sequences of the two isolates are available at the GenBank database with accession number PP422969 and PP422970 for STWF1 and LTWF1 respectively. These are available at the following URL: https://www. ncbi.nlm.nih. gov/nuccore/PP422969.1/ for STWF1 and https://www. ncbi.nlm.nih.gov/nuccore/PP422970 for LTWF1.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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