

Screening and molecular identification of biosurfactant-producing endophytes from *Quillaja lancifolia* D. Don

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Abstract

Endophytes are widespread microorganisms that colonize plant tissues without causing apparent harm. These organisms possess diverse metabolic repertoires and can be alternatives or complementary sources of high-value bioactive compounds. Saponins stand out due to their surfactant, foaming, and immunoadjuvant properties. Triterpene saponins from *Quillaja* spp. are used to enhance both humoral and cellular responses in human and veterinary vaccines. Herein, screening and identification of biosurfactant-producing endophytes from *Q. lancifolia* tissues were carried out. The aim of the study was to contribute to the characterization of endophyte microbial and biochemical diversity in *Q. lancifolia*. A total of 42 endophytes (22 fungi and 20 bacteria) were isolated from *Q. lancifolia* plantlets, callus, cell suspensions and seeds, followed by microbial culture, and screening for extracellular foam production. Four fungi isolates were selected as foam-producing strains and were taxonomically identified using ITS-1 DNA sequencing. Foam production tests were performed using extracts from the liquid filtrate of cultures for subsequent triterpene screening by thin layer chromatography. Select extracts were purified and concentrated by solid phase extraction. Antibacterial activity was screened through agar diffusion and microdilution tests. *Diplodia* sp., *Acremonium* sp., *Fusarium* sp. and *Aureobasidium* sp. were identified as natural foam-producing endophytes. Liquid chromatography-mass spectrometry analyses revealed major differences between plant and fungi purified fractions. This work constitutes the first report of endophytic microorganisms associated with *Q. lancifolia*. It not only provides insights on the metabolic potential of these beings but also buttresses future investigations on their biotechnological applications.

Highlights

- This is the first report of endophytic microorganisms in
- A total of 42 cultured endophytes were screened for extracellular foam production
- sp., sp., sp. and sp. yielded foam
- Fungal biosurfactants differed markedly from those of the plant host

Introduction

Quillaja lancifolia D. Don, synonymous with *Quillaja brasiliensis* (A.St.-Hil. & Tul.) Mart., is a tree species indigenous to Brazil, Uruguay, and Argentina. It is known as a natural source of triterpene saponins that can be used in the production of pharmaceuticals, food, and cosmetics (Luebert, 2014; Fleck et al. 2019).

Like the stem bark of *Q. saponaria* Molina, the leaves of *Q. lancifolia* produce triterpene saponins derived from quillaic acid, known for their notable immunoadjuvant properties (De Costa et al. 2011). Mechanistically, *Quillaja* spp. saponins enhance and prolong cellular and humoral immune responses as

adjuvants in various vaccines, including Polio, Zika, SARS-CoV-2, and Influenza viruses (De Costa et al. 2014; Cibulski et al. 2021; Kumar et al. 2022; Silveira et al. 2023).

Saponins, a class of bioactive compounds, exhibit surfactant properties, generating persistent foam in water, and consist of steroidal (C27) or triterpene (C30) structures (Ferraiuolo et al., 2022). Within *Quillaja* triterpene saponins, quillaic acid (C₃₀H₄₆O₅) serves as the principal aglycone, contributing to the formation of immunoadjuvant saponins like QS-21 (C₉₂H₁₄₈O₄₆). This saponin is approved for human vaccine adjuvant use, being primarily sourced from *Q. saponaria* bark. However, QS-21 is also found in *Q. lancifolia* leaves, being a component of an immunoadjuvant saponin-enriched fraction named QB-90 (Magedans et al. 2019) or its equivalent, fraction B (Wallace et al. 2019).

The unsustainable nature of commercially extracting these compounds from natural *Quillaja* populations has spurred interest in bioprospecting alternative sources for triterpene saponins production within the biotechnological sector. Current commercial strategies include cloning of superior tree genotypes in plantations and miniclinal gardens (Magedans et al. 2019).

Endophytic microorganisms possess the unique capability to inhabit plant tissues without inducing pathogenicity under homeostatic conditions (Petrini, 1991; Gupta et al. 2019), even in *in vitro* culture systems. The specialized metabolism of these endophytes represents a promising source of bioactive compounds (Gakuubi et al. 2021; Numan et al. 2022), owing to the inherent metabolic diversity of each species and the interaction with specialized metabolic pathways from their host plants. This symbiotic relationship can offer advantages such as differential growth, protection against various stresses and diseases, and chemical elicitation of specialized metabolism (Rabiey et al. 2019; Khan et al. 2021; Wu et al. 2021).

Microorganisms exhibit biotechnological potential for sustainable and scalable production of specialized metabolites from plants (Wawrosch and Zotchev, 2021). Consequently, exploring endophytic microorganisms associated with *Q. lancifolia* presents a potential new avenue for discovering strains capable of producing biosurfactants with significant biological activities. The primary aim of this study was to isolate and characterize the main endophytes of *Q. lancifolia* and examine their biotechnological potential as new sources of bioactive compounds, focusing on biosurfactant-like molecules.

Material and methods

Plant material and isolation of microorganisms

Seeds of *Q. lancifolia* were obtained from field-grown plants located in the rural area of Canguçu, Rio Grande do Sul, Brazil (31°05'14.6"S, 52°50'02.0"W) in April 2023. Genetic heritage access was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), under registration number A98B7B1. Plant vouchers were deposited in the ICN Herbarium of

the Botany Department at the Federal University of Rio Grande do Sul (identification numbers ICN 207362, 207363, and 207367).

To ensure asepsis and eliminate epiphytic microorganisms from the seed coat surface, a disinfection procedure was carried out using commercial antifungal Dithane® at 0.5% for 20 minutes, followed by washing with 70% ethanol for 1 minute and a subsequent wash with a 2% active chlorine sodium hypochlorite solution for 10 minutes. Following asepsis, the seeds underwent three rinses with sterile distilled water and were then placed in individualized vials containing 5 mL of sterile culture medium (0.5 × Murashige and Skoog medium, pH 5.8, 0.7% agar). The flasks were maintained in an in vitro cultivation room at 22 ± 2 °C, photoperiod of 16 hours, and an average irradiance of 30 μmol m⁻² s⁻¹.

After 14 days from sowing, fungi and bacteria were isolated from surface-disinfested *Q. lancifolia* ungerminated seeds. Seedlings from the germinated seeds were submitted to a new surface disinfection with 0.1% HgCl₂ for 10 minutes before endophyte growth induction (Boiu-Sicuia and Cornea, 2019). Microorganisms that became visible up until 14 days were isolated. Endophytes were also directly isolated from previously established *in vitro* cultures of calli and cell suspensions of *Q. lancifolia*.

Cultivation conditions of isolates

Bacterial isolates were cultivated in Tryptone Soy Broth (TSB) medium, a nutrient-rich medium that supports bacterial growth and promotes the production of extracellular metabolites (Arivo et al., 2023; Linh and Duc, 2025), while fungal isolates were cultivated in Czapeck medium (Guimarães et al., 2009), both media at pH 7. For liquid culture, sterile flasks (100 mL) were inoculated and incubated at 28 °C under constant agitation (150 rpm), for both fungi and bacteria. After cultivation for 5 days (bacteria) or 7 days (fungi), the culture broths were collected, centrifuged at 1,720 g for 30 minutes at room temperature, filtered, and freeze-dried (Guimarães et al., 2009; Jayasekara et al., 2022). The resulting powder was used for biosurfactant-like screening through differential foam formation test and Thin Layer Chromatography (TLC) analyses focusing on the detection of triterpenes.

Biosurfactant-like screening

For the foam formation test, solutions of lyophilized extracts were prepared at a concentration of 500 mg/mL in ultrapure water. Following vigorous manual shaking, these solutions were visually compared with the corresponding sterile nutrient broth at an identical concentration (based on the guidelines of the Brazilian Sanitary Vigilance Agency - Agência Nacional de Vigilância Sanitária, 2019).

For Thin Layer Chromatography, C18 silica plates were the stationary phase. BAW (butanol:acetic acid:water, 10:2:8, v/v/v) was the mobile phase. Samples were applied and run in a chromatographic chamber for 40 minutes. To identify the presence of triterpenes, plates were developed with sulfuric anisaldehyde, followed by drying at 105°C. Both *Q. lancifolia* leaf extract and *Q. saponaria* Quil-A fraction were used as standards. Bands that stained purple, blue, and green were considered positive for

triterpenes; media extracts yielding bands with Rfs comparable to those of plant extracts were also considered of interest for the study (Agatonovic-Kustrin et al. 2019; Marques et al. 2023).

Metabolite purifications

For Solid Phase Extraction (SPE), samples were loaded in C18 silica columns (5g), followed by elution with increasing methanol:water gradients (0, 10, 20, 30, 40, 50, 60, 70, and 100%). This procedure allowed the concentration of target compounds and removal of non-target ones. The 70–100% methanol fractions were lyophilized and weighed for quantification.

Equation 1 was employed to determine the yield of biosurfactant-like agents.

$$S (\%) = \frac{SPEm \cdot 100}{ce}$$

1

Where $S (\%)$ is the yield of total biosurfactant-like agents, $SPEm$ is the mass of purified extract after SPE extraction and ce is the mass of crude extract loaded into the SPE column.

Molecular Identification of Select Endophytes

DNA Extraction

For the extraction of DNA from select filamentous fungi, strains initially underwent cultivation on solid PDA medium in Petri dishes at 28 °C. Young mycelia were obtained from colonies after 7 days. In 2 mL microtubes, 100 mg of mycelium, and 1 mL of extraction buffer (100 mM Tris-HCl (pH 7.5), 25 mM EDTA, 1.5 M NaCl, 2% (w/v) CTAB - cetyltrimethylammonium bromide) at 65 °C were added, and the mixture was macerated using a Beader with 0.2 mL of sterile glass pearls for 1.5 minutes at 3,000 rpm.

Subsequently, β -mercaptoethanol was added to a final concentration of 3% (v/v), followed by incubation at 65 °C for 30 minutes in a water bath. Samples were then centrifuged for 5 minutes at 5,000 x g, the supernatant was collected into a new tube and partitioned with chloroform:isoamyl alcohol (24:1, v/v). The DNA, treated with RNase A for 15 minutes at 37 °C, underwent another partition with chloroform:isoamyl alcohol (24:1, v/v). The DNA was then precipitated with 95% ethanol for approximately 1h at -20°C and subsequently washed with 70% ethanol. After drying, the pellet was resuspended in TE buffer (100 mM Tris-HCl pH 7.5 and 25 mM EDTA). The final DNA concentration was adjusted to 15 ng/ μ L.

Polymerase Chain Reaction (PCR)

For PCR reactions, recombinant Taq DNA polymerase kit (Invitrogen, Waltham, MA, USA) was used. Sterile 0.2 mL microtubes were prepared containing 10 µL of 10X buffer, 3 µL of 50 mM MgCl₂ solution, 2 µL of 10 mM dNTP mixture, 5 µL of 10 µM primer mixture (F and R), 0.2 µL of recombinant *Taq* DNA Polymerase (Invitrogen), and 5 µL of DNA template at a concentration of 15 ng/µL.

The ITS-1 oligonucleotides were employed for the taxonomic identification of the fungi (ITS1-1F-F 3'-CTTGGTCATTTAGAGGAAGTAA and ITS1-1F-R 3'-GCTGCGTTCTTCATCGATGC) (White, 1990). The final reaction volume was 25 µL, and PCR was conducted over 35 cycles, involving denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min and 30 s. The amplification process commenced with an initial denaturation step of 3 min at 95 °C and ended with a final extension step of 5 min at 72 °C. Subsequently, the amplified products were subjected to electrophoresis in a 0.8% agarose gel, using FluoSafe as a nucleic acid dye. Electrophoresis was conducted at 100 mA for 45 minutes and bands were visualized under UV light.

DNA Sequencing and Data Analysis

Sequencing was carried out utilizing the Sanger sequencing method. The resulting data were compiled and utilized for species identification through the Basic Local Alignment Search Tool (BLASTn) with a public library available at <https://www.ncbi.nlm.nih.gov/>. Sequences for phylogenetic analysis were also obtained from the same database. The obtained DNA sequences were deposited in the NCBI database under accession numbers PX072001-PX072004 (Supplemental data S1).

Phylogenetic Analysis

To construct the phylogenetic tree, a data matrix was generated based on the lowest E-value results obtained from the NCBI's BLASTn tool (<https://blast.ncbi.nlm.nih.gov/>) for our sequences. The data matrix in .FASTA format underwent multiple sequence alignment using MAFFT, followed by an Alignment Curation using BMGE, a Tree Inference by PhyML + SMS and Tree Rendering by Newick Display (<https://ngphylogeny.fr/>) (Lemoine, et al.2019). Tree visualization and editing was performed using iTol (<https://itol.embl.de/>) (Letunic and Bork, 2021).

HPLC and LCMS

Purified fractions obtained from *Diplodia* sp. and *Aureobasidium* sp. were analyzed for presence of QS-21 and related saponins. High-Performance Liquid Chromatography (HPLC) was carried out using an external standard curve prepared with authentic QS-21 (Creative Biolabs, NY, USA) or reference QB-90 purified from *Q. lancifolia* leaves. Mobile phase consisted of a combination of acetonitrile (phase A) and Milli-Q water (phase B), both containing 0.1% formic acid. Gradient started at 25% of B changing to 50% of B within 20 min. A C18 RP column (250 mm x 4.6 mm (5µm) – Agilent Eclipse - XDB) was the stationary phase. Analyses were carried out at 214 nm in a Shimadzu Prominence chromatograph equipped with UV-Vis DAD detector.

Liquid Chromatography-Mass Spectrometry (LCMS) measurements were performed using the LCMS Amazon speed Iontrap (Bruker Daltonics Inc., MA, USA), following a previously established protocol (Wallace et al. 2019). Samples were injected at a concentration of 10 mg/mL (20 μ L) for preliminary evaluation. Authentic QS-21 (Creative BioLabs, NY, United States) was used as standard. Additionally, *Q. lancifolia* leaf extract was injected at 10 mg/mL to serve as a reference control for triterpene saponins. The liquid chromatography was conducted using a C18 column (250 mm x 4.6 mm, 5 μ m). The mobile phase consisted of water with 0.1% formic acid (v/v) for phase A and acetonitrile with 0.1% formic acid (v/v) for phase B. The gradient began with 15% B, increasing to 65% B over 50 minutes. The flow rate was maintained at 0.6 mL/min. Spectral data were collected in negative mode, with a full scan performed from 800 to 2500 m/z at a resolution speed of 8.1 m/z per second. The most abundant ion was isolated in the Ion Trap for MS2 fragmentation. MS2 data were manually deconvoluted to identify the target compounds.

Antimicrobial activity

The antimicrobial activity of fungal extracts, as well as crude and purified fractions of *Q. lancifolia*, was assessed using the agar diffusion method. Briefly, standardized inoculum at 0.5 Mc Farland scale (OD_{600} 0.08–0.1) of *Staphylococcus aureus* ATCC 35591, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 23893 were plated on TSA (Tryptone Soy Agar) using a sterile swab. Then, 20 μ L of each extract or disk with standardized antimicrobial were directly applied to the surface of agar plates. Petri dishes were kept at 37 °C for 24 hours, until inhibition zones were measured (Kimura et al. 2006; Motta and Brandelli, 2002).

Based on agar diffusion tests, select bacteria (*P. aeruginosa*) and extracts (SPE *Diplodia* and QB-90) were submitted to the microdilution test using the 96 well plate in TSB. Concentrations ranged from 2.5–0.001 mg/mL for purified *Diplodia* extract fraction, and from 25–0.012 mg/mL for QB-90. Chloramphenicol was used as standard drug (320 to 0.15 μ g/mL). After incubation, OD_{600} was recorded for each well for growth assessment. Percentage inhibition was calculated as response parameter for anti-*Pseudomonas* activity (adapted from Methodology for Agent Sensitivity Tests Antimicrobials by Dilution for Bacteria Aerobic Growth, M7-A6 standard, by NCCLS - National Committee for Clinical Laboratory Standards).

Results and discussion

Isolation and selection of microorganisms

Twenty-two fungal and twenty bacterial strains were isolated from *Q. lancifolia*. Among these, four fungal strains were selected based on their capacity to produce biosurfactants, as evidenced by differential foam formation and the presence of bands stained on TLC (strains 12PFR4, 06SFR4, and 12PFR1) using sulfuric anisaldehyde. No bacterial strain showed intense foam formation. Additionally,

fungal strain 22PFR4 (2) exhibited bands like some of the those of *Q. lancifolia* as assessed by TLC (Table 1, Fig. 1), albeit apparently not of triterpene nature.

Table 1
General characteristics of isolates positive for biosurfactants in foam and/or TLC tests.

Strain	Foam	TLC	Yield (% w/w)	Colony	Source
F5_12PFR4 (1)*	++	Green/purple	3.0	Grey/black	Seedling
F9_22PFR4 (2)	+	Yellow	0.74	Grey/black	Seedling
F21_06SFR4 (3)	+	Bluish pink/purple	0.40	White/pink	Seed
F23_12PFR1 (4)	++	Yellow/pink	6.28	Black/brown	Seedling
*(1) <i>Diplodia</i> sp.; (2) <i>Acremonium</i> sp.; (3) <i>Fusarium</i> sp.; and (4) <i>Aureobasidium</i> sp.					

Molecular Identification and Yield

Taxonomic identification conducted by sequencing of the ITS-1 region and local alignment analysis revealed the presence of *Diplodia* sp. (12PFR4), *Acremonium* sp. (22PFR4), *Fusarium* sp. (06SFR4), and *Aureobasidium* sp. (12PFR1). The total exuded fraction for these species was quantified using a triterpene saponin-specific purification method, resulting in yields of 3.0%, 0.74%, 0.40%, and 6.28% (w/w), respectively (Table 1). The macro and micromorphology of the colonies of the selected species are shown in Fig. 2.

In related studies focused on the bioprospecting of endophytes with saponin production, species such as *Aspergillus terreus*, *A. flavus*, *Penicillium* sp., and *Talaromyces pinophilus* have been isolated from the roots of *Asparagus racemosus* Willd., a saponin-producing plant naturally occurring in Africa, Asia, and Oceania (Rani *et al.* 2022). Besides, *F. oxysporum* and *A. niger* have been prospected from *Panax notoginseng* (Burk), which is naturally rich in ginsenosides. Saponins similar in character to those of the host plant have been elucidated, and the purified metabolites exhibited antimicrobial activity (Jin *et al.* 2017). Moreover, *F. tricinctum*, *Alternaria* sp., *Chaetosphaeronema* sp., *Mucor* sp., and *Trichoderma* sp. were isolated from *Lithospermum officinale* L., a plant producing shikonin - an anticancer compound (Mollaei *et al.* 2019). The same study also recorded this alkaloid in the endophyte *F. tricinctum*, suggesting the fungal strain could be able to produce plant-like specialized metabolites.

Phylogenetic Analysis

The phylogenetic tree illustrates the grouping of the select strains into four distinct clades. These separate groups represent the different genera, *Aureobasidium* sp., *Diplodia* sp., *Acremonium* sp. and *Fusarium* sp. (Fig. 3).

Diplodia sp., (Fig. 2 – a, b), appeared to be a predominant endophytic fungus in *Q. lancifolia*, as evidenced by its consistent re-isolation during the *in vitro* processing of cells and tissues from the same species. *Diplodia* belongs to the order Botryosphaerales and is primarily associated with diseases in

woody plant species and major crops, such as maize (Yang et al. 2017). This genus is affiliated with Rosaceae, and its species may or may not induce pathological conditions, like stem rot and leaf spots (Philips et al. 2012). *Diplodia* is considered a facultatively associated taxon with pathosystems but is also conclusively identified as a component of plant endophytic communities (White et al. 2016). Plant-associated *Diplodia* sp. have been shown to produce an array of specialized metabolites, mainly polyketide - and terpenoid - derived compounds. The most notable bioactivities of these fungi are antimicrobial and phytotoxic (Salvatore et al. 2025).

Despite some species of *Fusarium* being recognized for their negative effects in crops, species isolated from endophytic communities of medicinal plants show considerable potential for obtaining bioactive products from specialized metabolism, including some related to those of the plant hosts (Mollaei et al. 2019). For instance, *Fusarium* sp. isolated from *P. notoginseng* yielded several ginsenosides (Wu et al. 2013; Jin et al. 2017). Moreover, *Fusarium* isolated from *Dioscorea nipponica* was identified as a species of significance for the biotransformation and diversification of saponins from *Paris polyphylla* (Chen et al. 2022).

Acremonium, known for its phylogenetic complexity, is a genus associated with food contamination and a causal agent of plant diseases (Teixeira and Machado, 2003; Summerbell et al. 2011). It includes strains with endophytic character, such as *Acremonium* sp., isolated from *Lilium davidii*, which exhibited plant growth stimulation and control activity against phytopathogenic species (Khan et al. 2021). In another study, *Acremonium strictum* showed the ability to biotransform ginsenoside Rb1 from *P. ginseng* (Chen et al. 2008).

Aureobasidium sp., a dimorphic fungus, is considered of interest in the biotechnology sector due to *A. pullulans* production of industrial enzymes, antimicrobials, single-cell proteins, pullulans, leamokines with antimicrobial and cytotoxic effects on tumor cell lines, iron-chelating siderophores, and β -glucans (Chi et al. 2009; Manitchotpisit et al. 2011; Prasongsuk et al. 2017). *A. pullulans* A11211-4-57 produced two new biosurfactants, pullusurfactans F and G. These myo-inositol lipids exhibited strong surfactant activity at 1.0 mg/L, making them suitable for cleaning applications (Kim et al. 2022).

Although the production of complex plant specialized metabolites by fungal endophytes has been often reported, there have been several legitimate challenges to the validity of some long-term results involving the topic, at least in the case of taxol (Gärditz and Czesnick, 2024; Stadler and Kolarik, 2024). Indeed, in the present work, QS-21 could not be detected in the endophyte cultures. In addition, preliminary attempts to amplify from fungal DNA a key gene of QS-21 biosynthesis encoding beta-amyrin synthase proved unsuccessful (data not shown).

HPLC and LCMS results

Purified fractions from *Diplodia* sp. and *Aureobasidium* sp. aqueous extracts produced foam in water. Therefore, these samples were chemically analyzed for the presence of triterpene saponins. Initial HPLC data suggested the presence of saponin-like molecules in fungal extracts with peak retention times

resembling those of QS-21 and related saponins found in plants. However, LCMS² did not confirm any triterpenoid saponins in fungi samples (Fig. 4). In contrast, the expected presence of QS-21 in *Q. lancifolia* extract was confirmed, as previously reported (Wallace, 2019).

Antimicrobial activity

Potential inhibitory activity of plant and fungal extracts was tested against pathogenic Gram-positive and Gram-negative bacteria. Agar diffusion tests showed bioactivity of SPE purified fraction from *Diplodia* (5 mg/mL) and *Q. lancifolia* (QB-90, 50 mg/mL) against *P. aeruginosa*, with inhibition zones of 14.3 and 11.4 mm in diameter, respectively. Norfloxacin (30 µg/mL), a standard drug against Gram-negative bacteria, presented an inhibition diameter of 14.3 mm (Supplemental data S2).

Maximum growth inhibition has been obtained from broth microdilution tests, with 51.4% for SPE fraction from *Diplodia* sp. at 2.5 mg/mL and 42.2% for QB-90 (*Q. lancifolia* purified saponin fraction) at 25 mg/mL (Supplemental data S3). Despite the presence of various peaks on *Diplodia* sp. purified fraction chromatogram, indicating the presence of various compounds, an activity of more than 50% of inhibition was recorded against *P. aeruginosa*, an important Gram-negative pathogenic bacterium. Hence, a synergistic mechanism of action might be possible.

Previous studies from endophytic fungi from *Kigelia africana* Lam. (sausage tree) showed remarkable anti-*Pseudomonas* activity of *Neofusicoccum luteum* fractions with minimal inhibitory concentration of 156.50 µg/mL after seven days of fungal cultivation, and 19.53 µg/mL after 30 days of static fermentation. A purified compound, a C16-terpene dilactone presented MIC value of 0.61 µg/mL, playing a role in the antimicrobial activity (Bodede et al. 2022).

Despite the inhibitory activity exhibited by the *Diplodia* purified fraction in the first fermentation batch, a second independent experiment, conducted under identical culture media, temperature, agitation, and incubation time, yielded a different HPLC profile (Supplemental Figure S4). Although maintaining foaming capacity relative to the culture media control, bioactivity assessment by agar diffusion assay revealed no inhibitory activity of the purified fraction of the second batch against *P. aeruginosa*. This variability of chemical profile and associated bioactivity is likely due to metabolic changes in endophyte cultures over time, particularly following isolation from plant tissues. In fact, this is not surprising given the specific microenvironments, nutrient and energy sources, signal exchange molecules, and impact of seasonal variations of the *in planta* conditions, all of which differ markedly from *in vitro* media cultivation. Even within the endophytic environment, the temporal and spatial dynamics of fungal composition, abundance and interaction with host plants is quite significant (Crasta and Raveesha, 2024; Ullah et al. 2025).

Conclusion

This is the first report of endophytic microorganisms in *Q. lancifolia*. Four strains of fungi producing biosurfactants-like foaming agents were prospected from different plant sources and identified by

molecular techniques, confirming the presence of *Diplodia* sp., *Fusarium* sp., *Acremonium* sp. and *Aureobasidium* sp. Despite the absence of key plant compounds in the most promising select endophytes, purified fractions showed overt foaming properties. Endophytic species of *Q. lancifolia* may constitute a platform to produce compounds of biotechnological interest.

Declarations

Declaration of generative AI and AI-assisted technologies in the writing process

During the drafting stage of the manuscript, the author(s) used OpenAI's ChatGPT in order to assist with language refinement, and organization of the text. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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CRediT authorship contribution statement

Fábio Antônio Antonelo: Conceptualization, Investigation, Methodology, Writing – original draft. **Eliane Zachert:** Methodology, Writing – review & editing. **Yve Verônica Magedans:** Methodology, Data curation, Writing – review & editing. **Anna Alves Yendo:** Methodology. **Cibele Tesser:** Methodology. Validation. **Alice Elvira Teixeira dos Santos:** Methodology. **Fernanda Cortez Lopes:** Investigation, Methodology, Validation, Writing – review & editing. **Marilene Henning Vainstein:** Resources. **Charley Christian Staats:** Writing – review & editing. **Juliana Morini Küpper Cardoso:** Writing – review & editing. **Arthur Germano Fett-Neto:** Investigation, Resources, Supervision, Project administration, Validation, Writing – review & editing.

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Figures

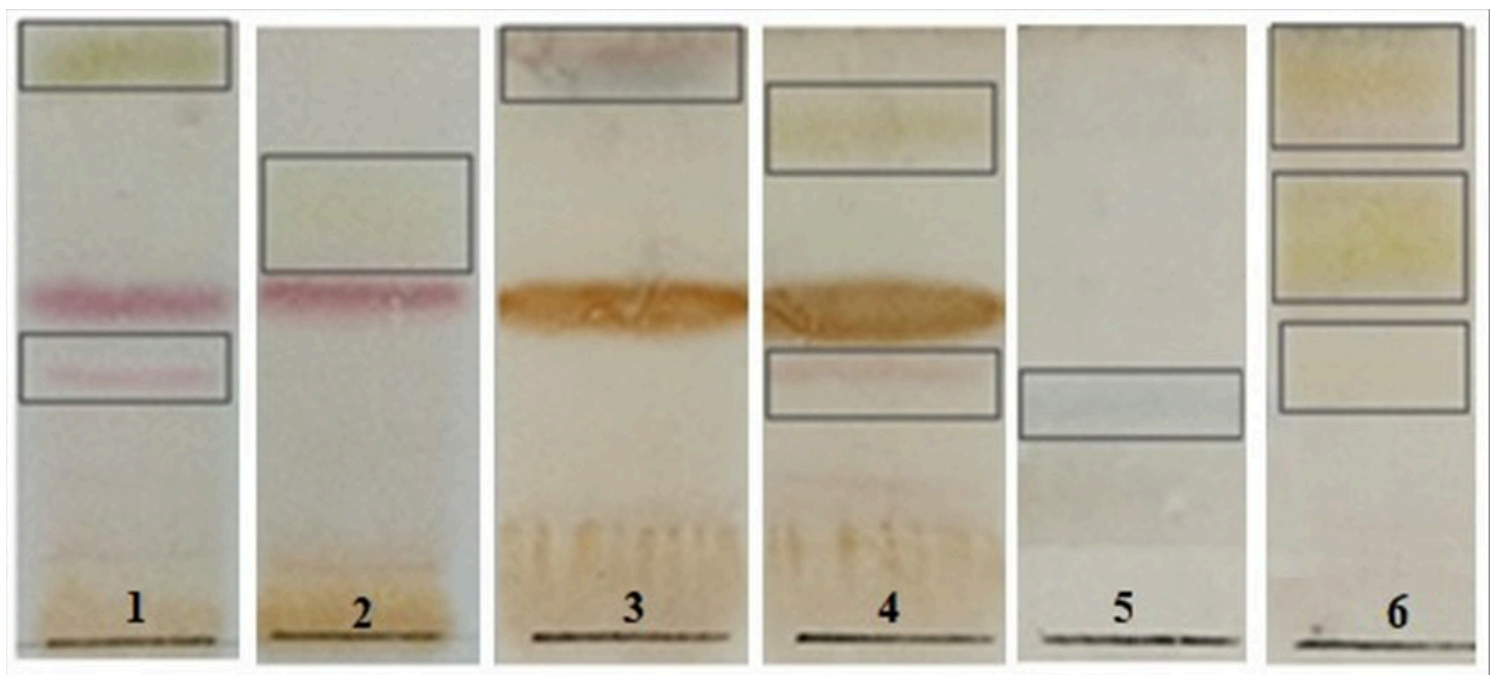


Figure 1

Thin Layer Chromatography of fungal and plant extracts. Fungal culture extracts ((1) *Diplodia* sp., (2) *Acremonium* sp., (3) *Fusarium* sp., and (4) *Aureobasidium* sp.), as well as extracts from *Q. saponaria* (5) and *Q. lancifolia* (6) were resolved in TLC plates. Triterpenes were visualized using sulfuric anisaldehyde.

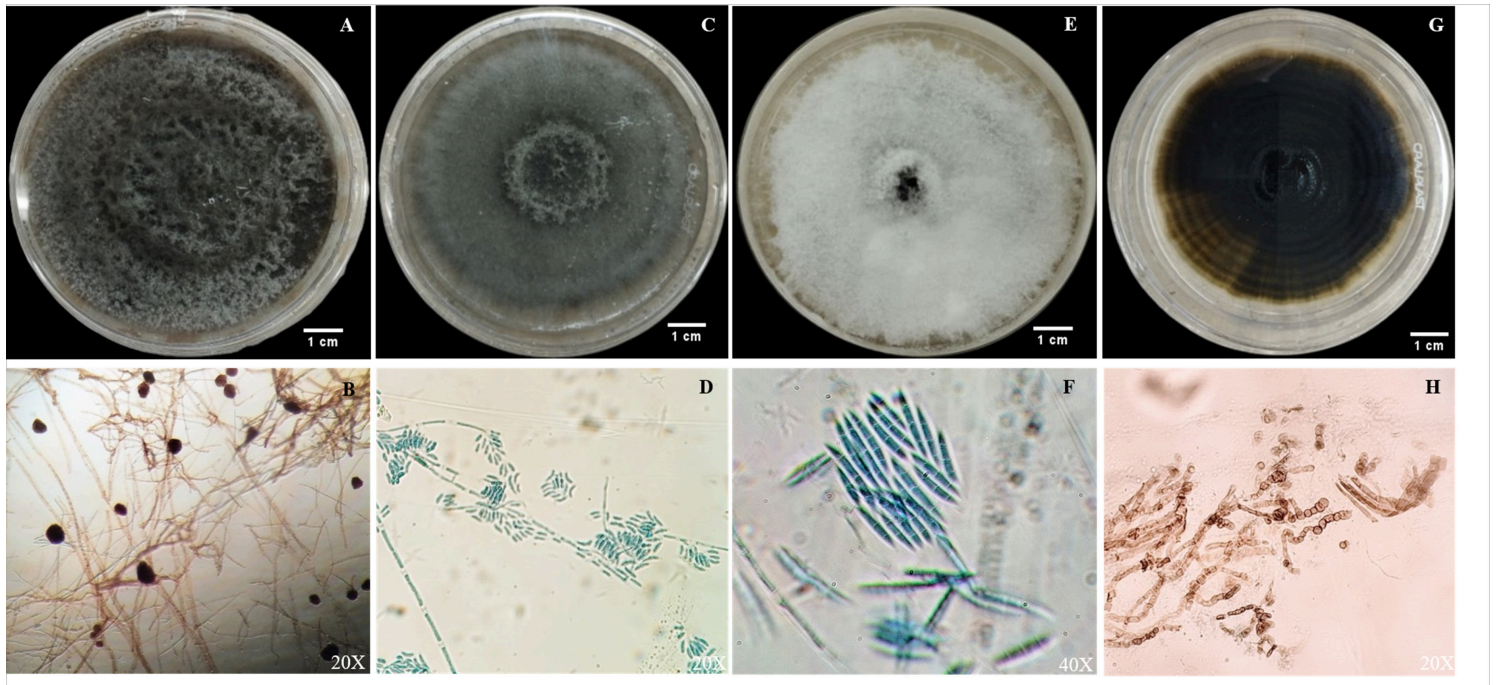


Figure 2

Macro and micromorphology of *Diplodia* sp. F5_12PFR4 (A, B), *Acremonium* sp. F9_22PFR4 (C, D), *Fusarium* sp. F21_06SFR4 (E, F), and *Aureobasidium* sp. - 12PFR1 (G, H)

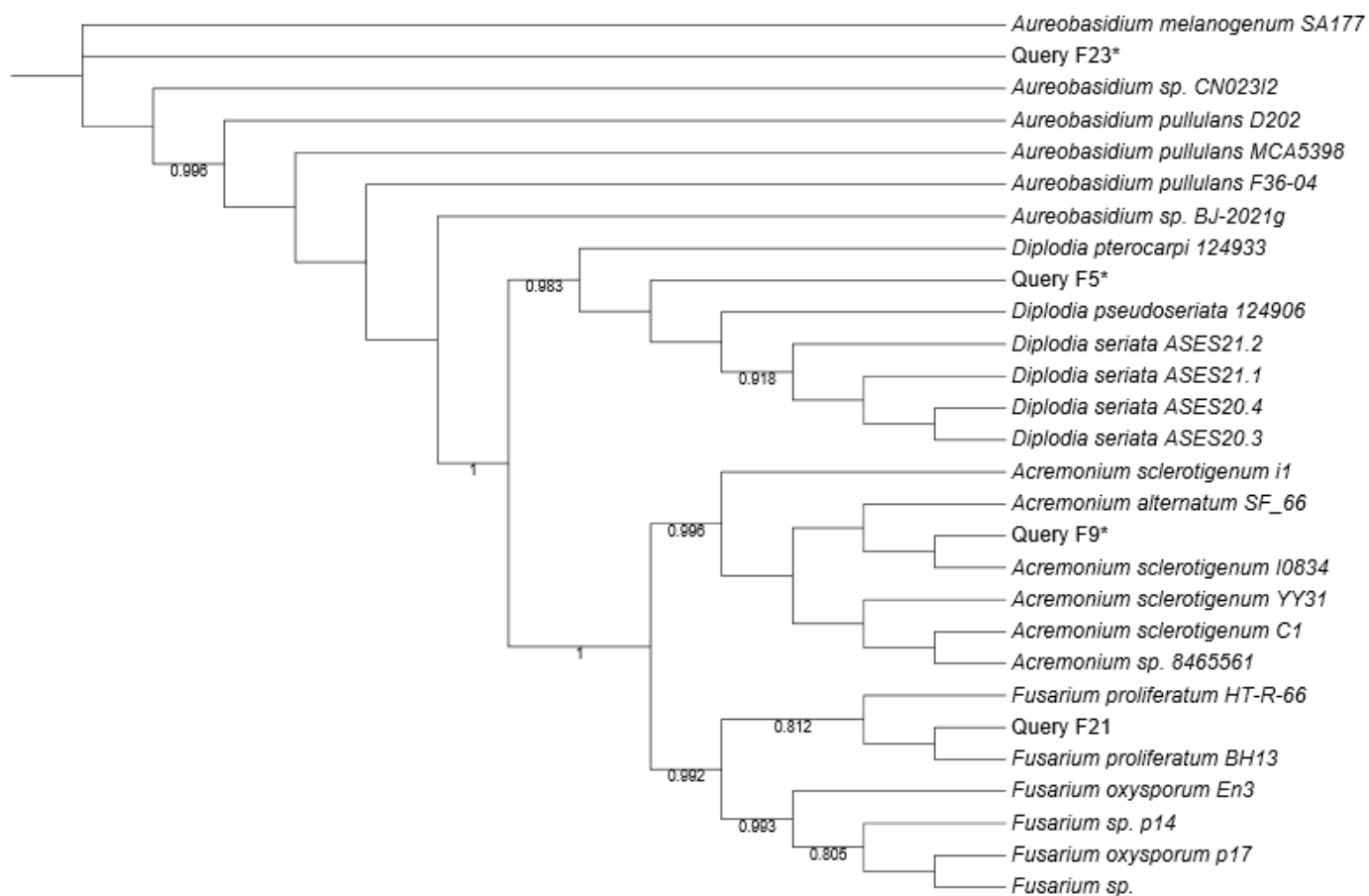


Figure 3

Phylogenetic tree of foam-producing endophytic isolates of *Q. lancifolia*.

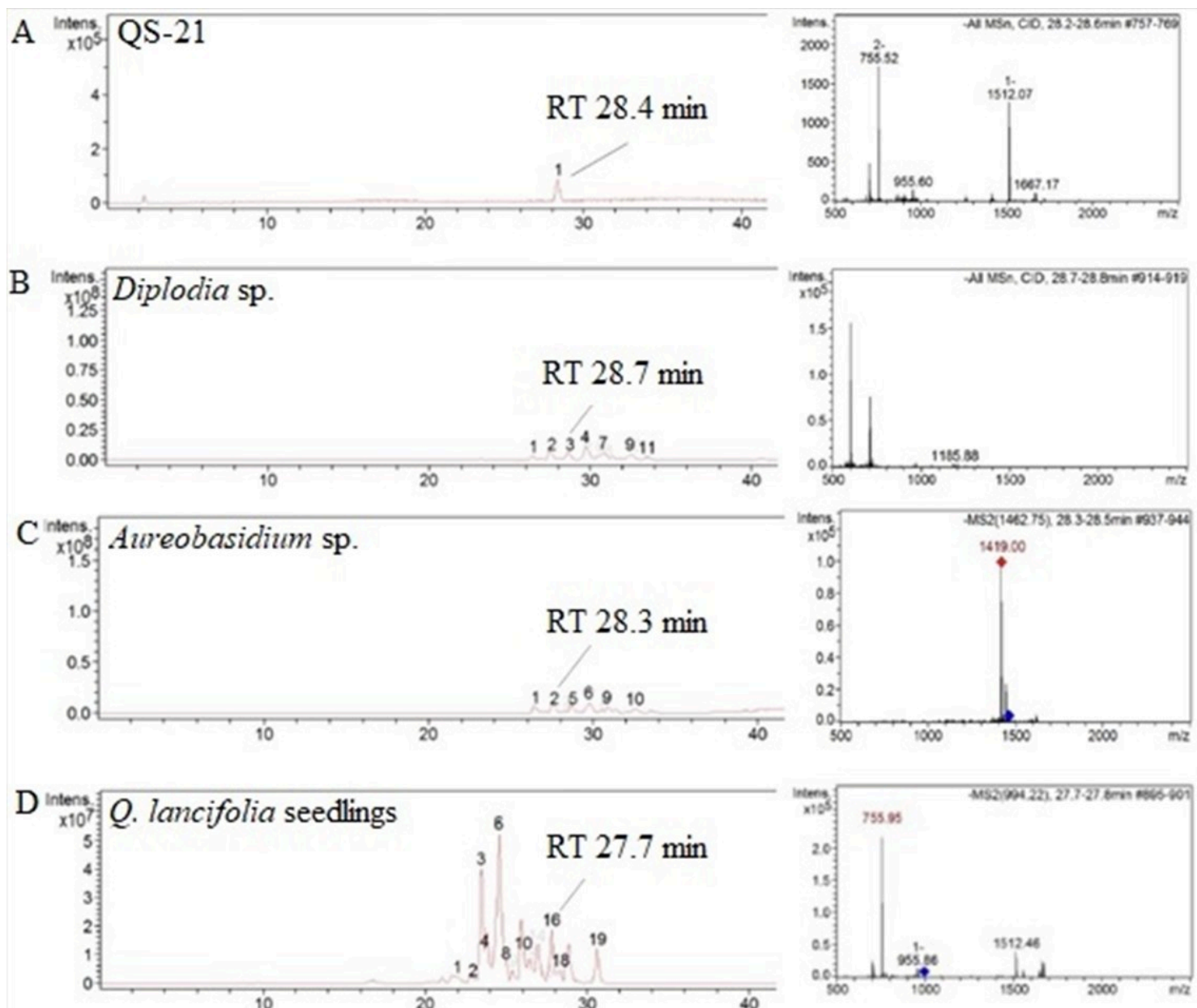


Figure 4

LCMS² and most abundant fragment from QS-21 standard (A), *Diplodia* sp. purified fraction (B), *Aureobasidium* sp. purified fraction (C) and *Q. lancifolia* seedlings purified fraction (D).

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