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New record of *Aureobasidium mangrovei* from plant debris in the Sultanate of Oman

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Aureobasidium mangrovei was isolated from plant debris in Muscat, Sultanate of Oman. The isolate was characterised and compared with related species of this genus for its growth, colony morphology, and micromorphology. Molecular analysis of the LSU and ITS rDNA supported final identification of the isolate. Our record is the second find in the world and the first in the Sultanate of Oman. DNA sequences of the isolated strain showed 99% (ITS) and 100% (LSU) similarity, respectively, with the sequences of the type isolates from Iran, as well as similar growth and colony morphology. A complete microscopic characterisation, which was not described for the Iranian strain, was made. The Iranian strains were isolated from saline habitats of the protected Hara forests, while our strain was isolated from the leaves of freshwater habitats. A comparison of growth characteristics of both strains under different conditions is provided.

Key words: Ascomycota, *Dothideales*, ITS, LSU, morphology, physiological characteristics, saprotroph.

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Aureobasidium mangrovei bylo izolováno z rostlinných zbytků v Maskatu (Omán). Izolát byl srovnán s příbuznými druhy po stránce růstu, morfologie kolonií a mikromorfologie; určení bylo potvrzeno molekulární analýzou LSU a ITS rDNA. Jde o druhý nález tohoto druhu na světě a první v Ománu. Sekvence DNA izolovaného kmene vykázaly 99% (ITS) and 100% (LSU) podobnost s typovým izolátem z Íránu, s nímž se shoduje i v charakteru růstu a morfologii kolonií. Je prezentována kompletní mikroskopická charakteristika, která doplňuje údaje z popisu kmene z Íránu. Zatímco íránské kmeny byly izolovány ze salinního prostředí v pobřežních porostech kolíkovníku, tento kmen byl izolován z listů ve sladké vodě. V práci je uvedeno srovnání růstových charakteristik obou kmenů.

Introduction

Aureobasidium is a genus of oligotrophic species of which *Aureobasidium pullulans* and *Aureobasidium melanogenum* have a global distribution and are ubiquitous on a variety of substrates such as soil, water, plant leaves, food stuffs, textiles, painted walls and moist inert surfaces (Moubasher et al. 2012). Several *Aureobasidium* species are known for their biotechnological and commercial significance, producing extracellular polysaccharides (EPS), hydrolytic enzymes, antimicrobial compounds, and biosurfactants (van Nieuwenhuijzen et al. 2016). The genus is currently classified in the family *Saccotheciaceae* of the order *Dothideales* (Wijayawardene et al. 2017, Nasr et al. 2018). Since its first description in the 19th century, numerous families have been included in and excluded from this order based on morphological and physiological characteristics. Molecular taxonomy research has confirmed part of the morphology-based classification (Wijayawardene et al. 2014). The family included four asexual genera, *Aureobasidium*, *Kabatiella*, *Pseudoseptoria* and *Selenophoma*, and three sexual genera, *Columnosphaeria*, *Pseudosydowia* and *Saccothecium* (Thambugala et al. 2014).

Aureobasidium pullulans is a classical, well-known species in *Aureobasidium*. Phenotypic varieties of this species were defined by Hermanides-Nijhof (1977) and subsequently studied by de Hoog & Yurlova (1994). Three varieties were proposed reflecting the species' morphological and physiological variability: *Aureobasidium pullulans* var. *pullulans*, *A. pullulans* var. *melanogenum* and *A. pullulans* var. *aubasidani* (Hermanides-Nijhof 1977, de Hoog & Yurlova 1994). Zalar et al. (2008) confirmed two of these varieties, var. *pullulans* and var. *melanogenum* by molecular analysis of parts of four genes [rDNA internal transcribed spacer (ITS), β-tubulin (*TUB*), translation elongation factor (*TEF1*), and elongase (*ELO*)], and added two additional varieties, var. *subglaciale* and var. *namibiae*. The rapid development of advanced sequencing and bioinformatics has deepened the understanding of evolutionary traits and increased the accuracy in the delimitation of these taxa. Gostinčar et al. (2014) used whole genome sequencing to study differences between the four varieties published by Zalar et al. (2008). They found that the varieties were significantly different and should be regarded as distinct species.

So far, a number of taxa have been characterised and identified as a species belonging to this genus such as *A. microstictum*, *A. proteae*, *A. pullulans* (incl. var. *aubasidani*), *A. lini*, *A. namibiae*, *A. melanogenum*, *A. leucospermi*, *A. subglaciale*, *A. iranianum*, *A. caulivorum* and *A. thailandense* (Zhang et al. 2019); more recently, four species were included into this genus: *A. mangrovei* (Nasr et al. 2018), *A. khasianum* (Prabhugaonkar & Pratibha 2018), *A. pini* (Jiang et al. 2019) and *A. tremulum* (Crous et al. 2019).

Aureobasidium mangrovei, described as a new species by Nasr et al. (2018), is a black yeast-like species from the leaves of mangrove trees in a marine habitat in Iran. Based on morphological, physiological and phylogenetic analyses of the large ribosomal subunit (LSU) and internal transcribed spacer (ITS), this species was found to belong to the genus *Aureobasidium*, but different from all currently known species, based on sequence divergence, cardinal growth temperature and salt tolerance. In the present study, we present a second record of *A. mangrovei* isolated from plant debris in a freshwater habitat in the Sultanate of Oman. The strain was identified based on two molecular markers (LSU and ITS). The aim of this paper is to provide a detailed description of morphology, growth characteristics and physiology, including a comparison with related species.

Material and methods

Strain studied. Plant debris was collected from the botanic garden at Sultan Qaboos University (SQU) in Muscat, Sultanate of Oman (coordinates: 23°35'22'' N, 58°9'56'' E), cut into small pieces aseptically and cultivated on Potato Dextrose agar plates (PDA: Carolina, Burlington, NC, USA) at 28 °C. The developing colonies were isolated and deposited into the reference culture collection of the Centraalbureau voor Schimmelcultures (housed at Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands) with accession number CBS 142331 (SARA-138H).

Macroscopic morphology and physiological characteristics. The isolate was transferred to fresh PDA, Malt Extract agar (MEA; as described by Nasr et al. 2018), Yeast Peptone Glucose agar [YPG: 10 g/l yeast extract (Titan Biotech, Bhiwadi, Delhi, India), 20 g/l peptone (Himedia, Marg, Mumbai, India), 20 g/l glucose (Himedia, Marg, Mumbai, India), and 15 g/l agar (Himedia, Marg, Mumbai, India)], Sabouraud Dextrose agar (SDA: Titan Biotech, Bhiwadi, Delhi, India), Czapek-Dox agar (CDA: Titan Biotech, Bhiwadi, Delhi, India), Yeast Extract agar (YEA: Titan Biotech, Bhiwadi, Delhi, India), and Harrold's M40Y and M60Y media as described by Raper & Fennell (1965). Morphological and physiological characteristics of the developing colonies were described. Colony diameters were recorded from 7-day old cultures. Four replicates were made. Surface colours of the growing colonies were rated using the colour Old Holland chart (2018). Photographs were taken from 14-day-old cultures grown on different media using a Canon 700D camera (Canon Inc., Ota, Tokyo, Japan).

Microscopic morphology. The strain was examined on PDA, being a medium proven to enhance fungal sporulation (de Hoog et al. 2019). Mounts of two-week-old slide cultures were prepared in lactic acid or lactophenol cotton

blue, and light micrographs were taken using a Nikon Eclipse 80i microscope with a Nikon digital sight DS-Fi1 camera (Nikon Instruments Inc., Melville, NY, USA). Spore dimensions were recorded, making 20 measurements for each spore type (hyaline, one-celled dark brown, two-celled dark brown, and chlamydospores). A drawing tube illustration was used to display the four morphological forms semi-diagrammatically and the drawings were inked using Rotring isograph pens of 0.18, 0.35, and 0.5 mm in diameter.

Molecular identification. DNA was extracted using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). The isolate was grown on MEA for two weeks at 28 °C. Cells were scraped off the colony surface with a loop, transferred to a bead solution, vortexed and treated following the manufacturer's protocol. Partial LSU was amplified by PCR with GeneAmp PCR System 9700 (Applied Biosystems, Waltham, MA, USA) using the primer pair LR0R (Rehner & Samuels 1994) and LR5 (Vilgalys & Hester 1990). The fragment of rDNA including ITS 1, 5.8S rDNA and ITS 2 was amplified by PCR with ITS1 and ITS4 primers (White et al. 1990). The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Purified PCR products were sequenced bidirectionally using the BigDye™ Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) and further purified using the BigDye® xTerminator™ purification kit (Applied Biosystems, Waltham, MA, USA). The samples were loaded on a 96 well-plate and run on a DNA sequencer 3730xl Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). Obtained forward and reverse sequences were aligned with ClustalW using the BioEdit 7.2.5 software (Hall 1999). The obtained nucleotide sequences were compared with the BLASTn search programme in GenBank to reveal similar sequence occurrences in the public database.

RESULTS AND DISCUSSION

Aureobasidium mangrovei S. Nasr, Antonie van Leeuwenhoek 111(9): 1701, 2018

Macromorphology. Species of the genus *Aureobasidium* have been shown to differ in their culture pigmentation (Zalar et al. 2008, Wang et al. 2019). *Aureobasidium mangrovei* SARA-138H grew in the first few days as pinkish colonies and became darkly pigmented after seven days of incubation (A74 ivory black extra) with a slimy and shiny appearance from the first week of incubation on YPG, SDA, PDA, CDA and YEA (Fig. 1). A yellow to orange layer (A319 Mars yellow) was observed at the margin of the cultures grown on YPG and YEA after 14 days of incubation. Similar pigmentation was observed on PDA and CDA during

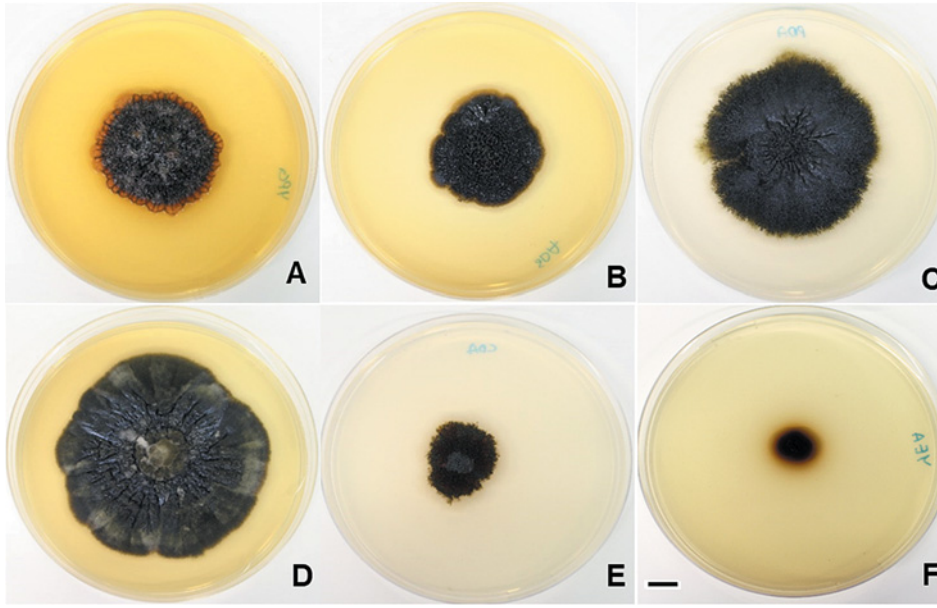

Fig. 1. *Aureobasidium mangrovei* (SARA-138H); 14-day-old colonies on different culture media incubated at 28 °C: **A** – YPG, **B** – SDA, **C** – PDA, **D** – MEA, **E** – CDA, **F** – YEA. Scale bar = 10 mm. Photo S. Al-Kindy.

the first week of incubation, but had disappeared in the 14-day-old cultures. On MEA, the fungus had an olive-green centre (A52 green earth) surrounded by a smooth black layer. In comparison, strains of *A. pullulans* and *A. subglaciale* remained pinkish for at least one week of incubation. Colonies of *A. pullulans* became entirely melanised after three weeks of incubation, while *A. subglaciale* remained pinkish for at least three weeks, with dark-pigmented hyphae developing at the margin. *Aureobasidium melanogenum* was characterised by an olive-brown to black centre with a yellowish margin. *Aureobasidium namibiae* was darkly pigmented with an olive-brown colour from the first week of incubation (Zalar et al. 2008). Of the five species of *Aureobasidium* (*A. pullulans*, *A. subglaciale*, *A. melanogenum*, *A. namibiae*, and *A. mangrovei*), the last three ones were characterised by an olive-brown to black centre, with a yellowish margin developing from the first week of incubation (Zalar et al. 2008, Nasr et al. 2018).

M i c r o m o r p h o l o g y. *Aureobasidium* species have a polymorphic life cycle consisting of hyphal growth and asexual sporulation, which were described and characterised by different researchers (Cooke 1962, Dennis & Buhagiar 1973, Hermanides-Nijhof 1977). Later, different terminologies were used to describe

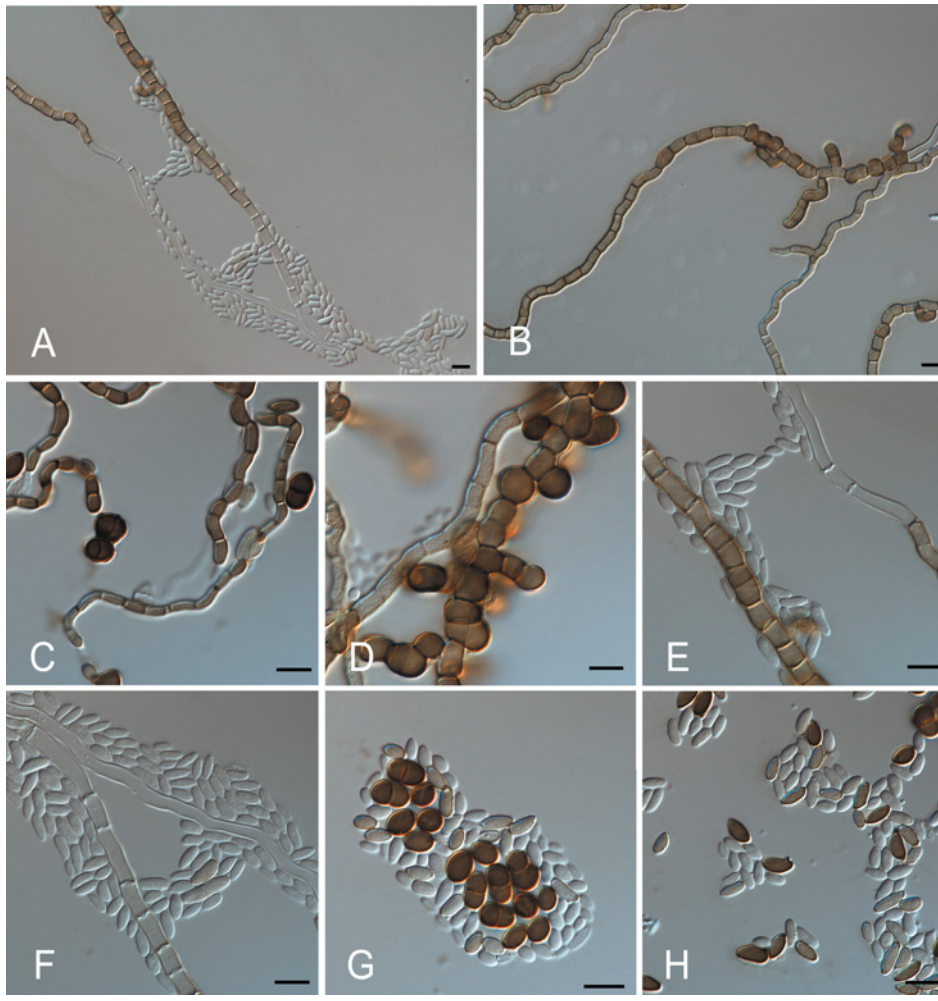

Fig. 2. *Aureobasidium mangrovei* (SARA-138H): **A** – hyphae surrounded by hyaline conidia, **B–D** – hyphae and chlamydospores, **E–F** – hyaline conidia, **G–H** – hyaline conidia, dark brown one- and two-celled conidia. Scale bars = 10 µm. Photo A. Al-Hatmi.

different developmental stages as follows: one-celled hyaline conidia (Zalar et al. 2008), budding blastospores (conidia) (Catley 1971), one- and two-celled brown conidia (Zalar et al. 2008), swollen cells (Simon et al. 1993, Li et al. 2009), chlamydospores (Simon et al. 1993, Zalar et al. 2008), endoconidia (Zalar et al. 2008). *Aureobasidium mangrovei* SARA-138H had hyaline, smooth, thin-walled, septate vegetative hyphae (Figs. 2, 3A). The one-celled hyaline conidia were smooth, ellipsoidal and variable in shape and size: 8.7–17.5 × 3.7–9.0 µm (Fig. 3B).

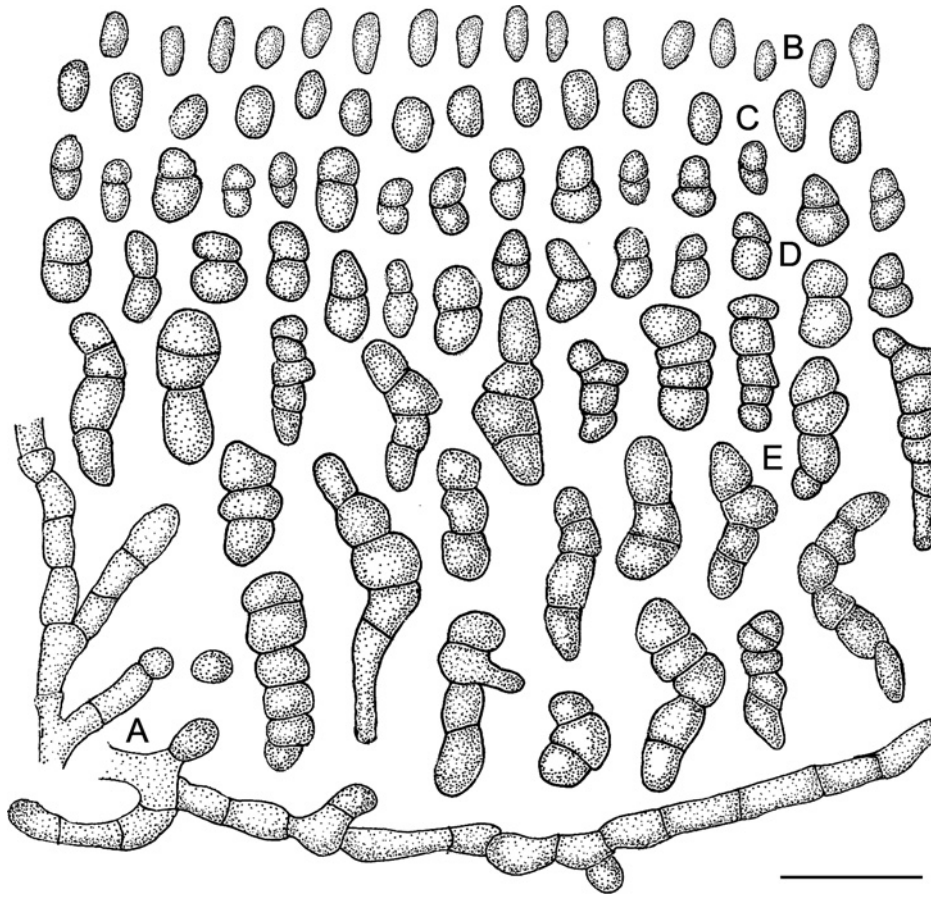

Fig. 3. *Aureobasidium mangrovei* (SARA-138H): **A** – mycelium, **B** – hyaline one-celled conidia, **C** – dark brown one-celled conidia, **D** – dark brown two-celled conidia, **E** – chlamydospores. Scale bar = 25 µm. Del. S. Al-Araimi.

Dark brown conidia were one- or two-celled. The size of the dark brown one-celled conidia was 6.3–17.8 × 5.8–11.0 µm (Fig. 3C). Two-celled dark brown conidia were slightly constricted at the septum, measuring 13.0–26.3 × 7.3–15.0 µm (Fig. 3D). Chlamydospores comprised three to nine cells and measured 22.5–99.8 × 8.0–13.5 µm (Fig. 3E). Budding of hyaline and brown conidia was frequently seen. Endoconidia not observed. Nasr et al. (2018) reported hyaline conidia of the Iranian *A. mangrovei* to be 7.0–13.5 × 3.8–9.0 µm in size, while those of *A. melanogenum* were 8–30 × 3.5–5.0 µm large, and those of *A. namibiae* 7–17 × 3.5–7.0 µm (Zalar et al. 2008). *Aureobasidium mangrovei* SARA-138H had hyaline conidia which were similar in size to those of *A. mangrovei* and *A. namibiae*.

Tab. 1. Physiological profiles of *Aureobasidium mangrovei* IBRC-M 30265 (Nasr et al. 2018) and *A. mangrovei* SARA-138H (CBS 142331) after 7 days of incubation at 28 °C.
Growth and temperature preferences are given as colony diameters (mm).

Growth conditions	*A. mangrovei* IBRC-M 30265	*A. mangrovei* SARA-138H
YPG	25	23
SDA	22	17
PDA	12	37
M40Y	26	60
M60Y	15	53
MEA + 5% NaCl	30	31
MEA + 10% NaCl	16	12
MEA + 15% NaCl	5	5
MEA + 20% NaCl	0	0
MEA + 25% NaCl	0	0
MEA at 4 °C	0	0
MEA at 15 °C	21	9
MEA at 25 °C	40	44
MEA at 28 °C	26	35
MEA at 34 °C	11	13
MEA at 37 °C	6	0
MEA at 40 °C	0	0

Molecular analysis. *Aureobasidium mangrovei* SARA-138H was identified using the ITS region and the D1/D2 domain of LSU, as described in Zalar et al. (2008) and Nasr et al. (2018). LSU sequences showed 100% similarity with and no single nucleotide polymorphism (SNPs) in the Iranian strains of *A. mangrovei* deposited in the GenBank database under accession numbers KY089084 and KY089086. The BLASTn analysis of ITS sequences showed 3 SNPs (99.25% similarity) for *A. mangrovei* IBRC-M 30265^{T} (KY089087) and 2 SNPs (99.43% similarity) for *A. mangrovei* IBRC-M 30266 (KY089085). The NCBI accession numbers for *A. mangrovei* SARA-138H are MK968773 (LSU) and MK968770 (ITS).

Physiology. The growth diameters of *A. mangrovei* IBRC-M 30265 and *A. mangrovei* SARA-138H (CBS 142331) under different growth conditions were compared (Tab. 1). Our strain CBS 142331 attained higher growth diameters on the media MEA, PDA, M40Y and M60Y compared with IBRC-M 30265. For salt tolerance, CBS 142331 and IBRC-M 30265 were similar, being able to tolerate up to 15% NaCl. CBS 142331 failed to grow at 4 °C, similarly to IBRC-M 30265. At 15 °C, CBS 142331 had a weaker growth compared to IBRC-M 30265. Strain IBRC-M 30265 attained a growth diameter of almost twice that of CBS 142331 at 15 °C. The optimum growth temperature for our isolate was 25 °C. The maximum tem-

perature for the growth of CBS 142331 was 34 °C, whereas IBRC-M 30265 was able to grow at temperatures of up to 37 °C.

E c o l o g y. *Aureobasidium* species exhibit oligotrophic and extremotolerant life styles as epiphytes and surface colonisers, occasionally as opportunistic human pathogens (Gostinčar et al. 2018, Nasr et al. 2018), while close relatives occur as endophytes. The Iranian strains of *A. mangrovei* were recovered from healthy mangrove plants (*Avicennia marina*) of the protected Hara saltwater mangrove forests on Qeshm Island. The hara tree (*A. marina*) is a saltwater plant which is submerged at high tide; part of the tree is seen above the saline water level, while the rest is below the water surface (Zahed et al. 2010). In contrast, *A. mangrovei* SARA-138H was isolated from plant debris in a botanical garden with a freshwater ecosystem.

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