

**SIMONE ALBINO PAES**

**MOLECULAR PHYLOGENY AND MORPHOLOGICAL CHARACTERIZATION  
OF THE ETIOLOGICAL AGENT OF SOUR ROT ON FRUITS AND VEGETABLES  
IN BRAZIL**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Doctor Scientiae*.

Orientador: Olinto Liparini Pereira

Coorientador: Ailton Reis

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Assentimento:



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Simone Albino Paes  
Autora



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Olinto Liparini Pereira  
Orientador

Dedico,

A Deus pela minha vida, e especialmente  
à minha família que soube entender  
minha ausência!

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*“Só a experiência própria é capaz de tornar sábio o ser humano.”*

Sigmund Freud

## RESUMO

PAES, Simone Albino, D.Sc., Universidade Federal de Viçosa, abril de 2020. **Filogenia molecular e caracterização morfológica do agente etiológico da podridão azeda em frutas e hortaliças no Brasil.** Orientador: Olinto Liparini Pereira. Coorientador: Ailton Reis.

A podridão azeda representa um problema sério no campo e na pós-colheita de frutas e hortaliças, limitando a produção e a comercialização. *Geotrichum candidum* tem sido relatado como a principal espécie causando esta doença. Neste estudo, 165 isolados causadores de podridão pós-colheita em diferentes estados do Brasil foram caracterizados com base na filogenia molecular e morfologia. Para caracterização molecular, o DNA genômico foi extraído, e o domínio D1/D2 do gene rRNA da subunidade maior (LSU) foi amplificado e sequenciado. As análises filogenéticas baseadas no domínio D1/D2 revelaram que 129 isolados pertencem a espécie *Geotrichum candidum*, enquanto outros isolados não agruparam com nenhuma espécie conhecida, provavelmente representando novas espécies. Além disso, outros gêneros, isto é, *Hyphopichia*, *Magnusiomyces*, *Saccharomycopsis*, *Trichosporon* e *Zygoascus*, foram identificados associados a podridão azeda em frutas e hortaliças no Brasil. Todas as espécies testadas, incluindo as possíveis espécies novas, foram patogênicas quando inoculadas artificialmente em frutos sadios de tomate do tipo longa vida, italiano e sweet grape, bem como em tubérculos de batata e frutos de seriguela. Em conclusão, embora *Geotrichum candidum* tenha sido relatado como a única espécie a causar podridão azeda, outras espécies de *Geotrichum* e outros gêneros estão associados à podridão azeda em frutas e hortaliças no Brasil, o que demonstra uma diversidade de fungos associados a essa doença.

Palavras-chave: Dipodascaceae. *Geotrichum*. Patologia pós-colheita. Podridões.

## ABSTRACT

PAES, Simone Albino, D.Sc., Universidade Federal de Viçosa, April, 2020. **Molecular phylogeny and morphological characterization of the etiological agent of sour rot on fruits and vegetables in Brazil.** Advisor: Olinto Liparini Pereira. Co-advisor: Ailton Reis.

Sour rot represents a serious problem in the field and postharvest of fruits and vegetables by limiting production and marketing. *Geotrichum candidum* has been reported as the main species causing this disease. In this study, 165 fungal isolates causing postharvest rot in different states of Brazil were characterized on the basis of molecular phylogeny and morphology. For molecular characterization, the genomic DNA was extracted, the D1/D2 domain of large subunit rRNA (LSU) gene was amplified and sequenced. The phylogenetic analyses revealed that 129 isolates of this study grouped with the species *Geotrichum candidum*, while other isolates did not cluster to any known species, probably representing new species. Moreover, other fungal genera, *i.e.* *Hyphopichia*, *Magnusiomyces*, *Saccharomyopsis*, *Trichosporon* and *Zygoascus* were identified associated with the sour rot disease on fruits and vegetables in Brazil. All species tested, including the putative new species, were pathogenic when artificially inoculated on healthy tomato fruit types, Long-self life, Italian tomato and sweet grape, as well as potato tubers and red mombin fruits. In conclusion, although *Geotrichum candidum* has been related as the only species causing sour rot, other species of *Geotrichum* and other genera are associated with sour rot on fruits and vegetables in Brazil, which demonstrates a diversity of fungi associated with this disease.

Keywords: Dipodascaceae. *Geotrichum*. Postharvest disease. Rot disease.

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## INTRODUÇÃO GERAL

Pelas suas condições edafoclimáticas e geográficas, o Brasil produz ao longo do ano uma grande variedade de frutas e hortaliças, desde aquelas originárias de clima tropical até as de clima subtropical e temperado (CENCI et al., 1997; ZUCOLOTO et al., 2017).

A produção aproximada de hortaliças no Brasil está em torno de 53 mil toneladas, com uma área cultivada de 5,1 milhões de hectares. O setor oferece cerca de 7 milhões de empregos diretos ou indiretos e movimenta anualmente um valor superior a 60 bilhões de reais (ANUÁRIO BRASILEIRO DE HORTI E FRUTI, 2019).

No setor de fruticultura, o Brasil possui uma área cultivada de 2,4 milhões de hectares, com uma produção estimada de 44 milhões de toneladas, gerando um valor superior a 40 bilhões de reais por ano. O setor frutícola é responsável pela geração de mais de 6 milhões de empregos diretos (ANUÁRIO BRASILEIRO DE HORTI E FRUTI, 2019).

Dentre os polos produtores de hortifruti que incluem locais tradicionais do setor como os estados de São Paulo, Minas Gerais e os do Sul, a área plantada está diminuindo, devido a uma menor disponibilidade de mão de obra, preço elevado das terras e problemas fitossanitários. Enquanto, o Cerrado e o Nordeste estão expandindo a produção, com destaque para Rio Grande do Norte, Ceará, Vale do São Francisco, Cristalina em Goiás, região do Irecê e da Chapada Diamantina na Bahia e o Norte e Triângulo Mineiro/Alto Paranaíba em Minas Gerais (ANUÁRIO BRASILEIRO DE HORTI E FRUTI, 2019).

Embora exista uma diversidade de produtos hortícolas e frutícolas para comercialização, ainda existe uma limitação quanto às vendas, pelo fato de serem altamente perecíveis em função do alto teor de umidade (COELHO et al., 2015). No Brasil, embora sejam produzidos e disponibilizados produtos hortícolas para uma parcela substancial da população, ainda é possível observar perdas desde os processos de colheita até o armazenamento (ALVES et al., 2010).

Em nível nacional são registrados altos níveis de perdas pós-colheita em cultivos de frutas e hortaliças, o que coloca o Brasil entre os 10 países que mais desperdiçam alimentos no mundo. Estima-se que 86% das perdas de frutas e hortaliças no Brasil ocorrem durante a exposição para vendas, 9% no transporte e 5% no armazenamento (MELO et al., 2013).

As perdas pós-colheita podem ocorrer devido a doenças, injúrias mecânicas e perdas fisiológicas (CHITARRA & CHITARRA, 2005). Pesquisas devem ser conduzidas para que essas perdas sejam minimizadas, aumentando a oferta de alimentos, além de evitar perdas de investimentos financeiros e de tempo gasto na produção. As injúrias mecânicas por si só, não

inviabilizam o consumo das frutas e hortaliças, mas reduzem as vendas devido ao dano estético e podem servir de porta de entrada a patógenos predispondo-as às doenças pós-colheita.

As perdas em pós-colheita são relacionadas principalmente a incidência de podridões causadas por microrganismos (CHITARRA & CHITARRA, 2005). Os fungos são responsáveis por 80% a 90% do total de perdas pós-colheita causadas por agentes microbianos (CAMARGO et al., 2011). Além das doenças presentes no campo, as que ocorrem em pós-colheita têm sido preocupação em relação a saúde do consumidor e vida útil do produto (CHITARRA & CHITARRA, 2005). A redução das perdas no pós-colheita representa um desafio constante.

O manejo das podridões em pós-colheita é de extrema importância para o sucesso da cadeia produtiva de frutas e hortaliças, pois a sua ausência pode inviabilizar a comercialização. Portanto, pesquisas voltadas à prevenção/controle de doenças em pós-colheita de frutas e hortaliças estão em pauta nos últimos anos. Embora a sintomatologia das podridões seja variável, pode-se destacar algumas nas quais os sintomas são semelhantes. Dentre elas incluem as podridões mole, seca, úmida e azeda.

A podridão azeda, tradicionalmente associada a espécies de *Geotrichum*, é atualmente um dos principais problemas em pós-colheita de frutas e hortaliças (HOOG & SMITH, 2004; AGRIOS, 2005; MCKAY et al., 2012). Embora *Geotrichum candidum* seja relatado como um dos agentes causadores de deterioração de vários alimentos, esse fungo é comumente inoculado em queijos com superfície amadurecida para alteração do sabor. Além disso, essa espécie é bastante estudada devido às características biotecnológicas, importantes para a indústria de alimentos (MARCELLINO et al., 2001; NAUMOVA et al., 2001; GENTE et al., 2002, 2006).

A podridão azeda caracteriza-se inicialmente por pequenas áreas macias com encharcamento e amolecimento do tecido, com posterior aparecimento dos sinais do patógeno, caracterizado pela formação de um micélio esbranquiçado na superfície do produto. O produto torna-se apodrecido, com odor etílico e impróprio para a comercialização e consumo. O fungo infecta frutas e vegetais através de lesões causadas por insetos ou ferimentos mecânicos (AGRIOS, 2005).

Similaridades morfológicas do gênero *Geotrichum* que ocorrem entre os isolados da mesma espécie e espécies distintas com morfologia semelhante são limitações para a identificação do gênero baseado somente em características fenotípicas (BUTLER et al., 1988; HOOG & SMITH, 2004; GROENEWALD et al., 2012). Somente as características fenotípicas não são suficientes para distinguir as espécies de *Geotrichum*, devido as espécies possuírem poucos caracteres morfológicos que possam ser comparados (PRILLINGER et al., 1999).

Apesar da crescente importância quanto à podridão azeda nos trópicos, a etiologia da doença é ainda fonte de controvérsia. Portanto, estudos relativos à caracterização do patógeno são necessários, para posterior estabelecimento de estratégias de manejo dessa importante doença pós-colheita.

Visando aumentar a precisão na identificação dos agentes etiológicos das podridões pós-colheita, técnicas moleculares, tais como uso de uma ou algumas regiões gênicas, têm sido utilizadas para descrever espécies de fungos fitopatogênicos além da caracterização morfológica (PIMENTA et al. 2005; SUH et al., 2006; WUCZKOWSKI et al. 2006; KWASNA et al., 2007; SULO et al. 2009; KAEWWICHIAN et al. 2010; NIELSEN et al. 2010).

Estudos iniciais com filogenia molecular de *Geotrichum* foram inferidos a partir de sequências das regiões ITS, 18S e 26S rDNA (UEDA-NISHIMURA et al., 2000; HOOG & SMITH, 2004; SUH et al., 2006). Porém alguns estudos revelaram que a região ITS é difícil de alinhar, curtas e com vários picos em única posição mesmo após várias tentativas de sequenciamento, dificultando a sua utilização para discriminação de espécies de *Geotrichum* (HOOG et al., 2004; ALPER et al., 2011; GROENEWALD et al., 2012). Devido a essas variações, a região ITS tem gerado incertezas em estudos de análises filogenéticas para identificação ou diferenciação de espécies de *Geotrichum* (HOOG & SMITH, 2004; GROENEWALD et al., 2012). Sequências do domínio D1/D2 da região LSU rRNA têm sido comumente utilizadas em estudos filogenéticos moleculares nesse gênero (SUH et al., 2006; GROENEWALD et al., 2012), uma vez que fornecem resolução satisfatória para identificação de espécies (KURTZMAN et al., 2013).

O conhecimento acerca da estrutura populacional de *Geotrichum* é necessário para desenvolver estratégias satisfatórias no controle da podridão azeda em frutas e hortaliças. Desta forma, o objetivo deste trabalho é elucidar a etiologia da podridão azeda em frutas e hortaliças no Brasil, por meio de análises filogenéticas utilizando o domínio D1/D2 da região LSU rRNA e morfologia dos agentes causais.

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## Molecular phylogeny and morphological characterization of the etiological agent of sour rot on fruits and vegetables in Brazil

### Abstract

The sour rot is an important postharvest disease that attacks fruits and vegetables in Brazil. *Geotrichum candidum* has been reported as the main species causing this disease. However, the identity of the causal agent is still uncertain. This research aimed to determine the identity of 165 fungal isolates associated with sour rot, obtained from fruits and vegetables in Brazil. The isolates were identified based on phylogenetic analyses, through the use of DNA sequences from the D1/D2 domain of the large-subunit (LSU) rRNA gene, and on morphological analyses. The phylogenetic analyses indicated that 129 samples of this study belong to the *Geotrichum candidum*, 15 to *G. citri-aurantii*, 6 to *G. phurueaensis*, 2 to *G. vulgare*, 1 to *Hyphopichia burtonii*, 1 to *Hyphopichia khmerensis*, 3 to *Saccharomycopsis crataegensis*, 1 to *Saccharomycopsis vini*, 1 to *Magnusiomyces tetrasperma*, 1 to *Trichosporon coremiiforme* and 1 to *Zygoascus meyeriae*. Besides, two putative new species were found, namely, *Geotrichum* sp. 1 (on potato) and *Geotrichum* sp. 2 (on red mombin). This study is the first to report the presence of five other genera besides *Geotrichum* associated with sour rot on fruits and vegetables, which demonstrates the diversity of fungi and yeasts associated with this disease. All genera identified were pathogenic when artificially inoculated on healthy tomato fruits, Longa Vida, Italiano, and Sweet Grape, including the new species when inoculated on potatoes and red mombins. The present work contributes to the knowledge about the diversity of fungi associated with sour rot disease and consequently to the selection of efficient control strategies to reduce the losses that cause postharvest damage on fruits and vegetables in Brazil.

### 1. Introduction

*Geotrichum* is a fungus genus with dimorphic colonial morphotypes (cream-colored yeast-like form or mold-like colonies) included in the family Dipodascaceae within the Saccharomycetales (ALPER et al., 2011; KURTZMAN et al., 2013). *Geotrichum* species are found in many substrates, including soil, plants, milk and water or associated with insects (HOOG & SMITH, 2004; SUH & BLACKWELL, 2006; ALPER et al., 2011). Some species are important postharvest plant pathogens of fruits and vegetables that cause economic losses in agriculture worldwide, due to the occurrence of sour rot in stored fruits and vegetables (HOOG & SMITH 2004; AGRIOS, 2005; MCKAY et al., 2012). These fungi infect fruits and

vegetables through injuries caused by insects or mechanical wounds. The symptoms initially appear as water-soaked and soft areas accompanied by a white-to-cream fungal growth on the surface tissues and a sour odor (AGRIOS, 2005).

*Geotrichum* is morphologically characterized by conidia (arthroconidia) formed in chains by the random fragmentation of the hyphae (HOOG et al., 1986). Species are difficult to distinguish due to the limitation of the phenotypic characteristics to the taxonomy of the genus (BUTLER et al., 1988; HOOG & SMITH, 2004; GROENEWALD et al., 2012).

The identification of *Geotrichum* is based on the combination of several techniques: morphology, physiology, ecology, mating type systems, DNA reassociation, PCR-fingerprinting and gene sequencing (NAUMOVA et al., 2001; HOOG & SMITH, 2004; GENTE et al., 2006; SUH & BLACKWELL, 2006; GROENEWALD et al., 2012).

*Geotrichum citri-aurantii* was reported only on citrus fruits and soil, while *G. candidum* occurs in various habitats, such as microflora of raw milk and cheeses, soil, plants, fruits, water and insects (HOOG & SMITH, 2004; GENTE et al., 2006; ALPER et al., 2011; MCKAY et al., 2012). It is a rare human pathogen that can colonize human skin, respiratory system and gastro-intestinal tracts, causing a disease referred to as geotrichosis (HENRICH et al., 2009; JAGIRDAR et al., 1981; NG et al., 1994; SHEEHY et al., 1976). The main risk factor for geotrichosis is immunocompromised (e.g. HIV, steroid/immunosuppressant use, diabetes mellitus, alcoholism and critical illness) (KEENE et al., 2019).

Although *G. candidum* causes deterioration of various foods, this fungus is commonly utilized by the commercial cheesemaking industry on surface-ripened cheeses for flavor change (ALPER et al., 2011). Additionally, this species is extensively studied for its biotechnological characteristics and importance for the food industry (MARCELLINO et al., 2001; NAUMOVA et al., 2001; GENTE et al., 2002, 2006).

*Geotrichum candidum* has been reported as the main species causing sour rot disease in Brazil. However, a comprehensive study to elucidate the etiology of this disease in the Brazilian conditions employing molecular techniques has never been conducted. Thus, the identity of the causal agent of sour rot is still uncertain. The correct identification of the etiological agents and knowledge of the association of the pathogen with the host provide efficient studies and techniques for management strategies of this important postharvest disease.

In the present study, the combined phylogenetic analyses with morphological characteristics were used to establish the identity of the isolates associated with sour rot in fruits and vegetables in Brazil. The etiology of the sour rot was investigated by comparing DNA

sequences from the D1/D2 domain of the large-subunit (LSU) rRNA gene, morphology characterization and pathogenicity test.

## 2. Material and Methods

### 2.1 Sampling and isolation

From 2014 to 2020, fruits and vegetables showing sour rot symptoms were collected from different hosts and Brazilian states (Table 1). The fungal isolates were obtained by direct transfer of spores to Potato Dextrose Agar (PDA) (Sigma- Aldrich, Spain) amended with the antibiotic tetracycline (20 mg L<sup>-1</sup>) in the laboratory. Monosporic culture was obtained to ensure the maintenance of the genetic identity of each isolate. For such, a serial dilution was performed and 20 µL of the spore suspension was transferred to a Petri dish containing 3% water agar (WA) (Vetec, Sigma-Aldrich, Brazil), spread with a Drigalski spatula and incubated at 25 °C, for about 12 hours. A single germinated conidium was transferred to plates containing PDA under a stereomicroscope [Motic® SMZ-168 (20X)]. The plates were incubated at 25 °C for 12h. All isolates were stored in sterile distilled water and in 2 mL microtubes containing 10% glycerol solution (CASTELLANI, 1939; GONÇALVES et al., 2016).

The isolates of the two new species of *Geotrichum* and one representative isolate of the species of *Geotrichum candidum*, *Geotrichum phurueaensis*, *Geotrichum citri-aurantii*, and *Geotrichum vulgare* were deposited in the culture collection of fungi “Coleção Octávio Almeida Drummond” (COAD). Metabolically inactive cultures were deposited in “VIC” Herbarium, both housed at the “Universidade Federal de Viçosa” in Viçosa-MG, Brazil.

### 2.2 DNA extraction, sequencing, and phylogenetic analyses

Genomic DNA was extracted from monosporic cultures grown on PDA plates covered with cellophane paper and kept at 25 °C, for 7 days. The mycelium was removed with sterile toothpicks and placed in 1.5 mL microtubes along with four steel beads. The genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega Corporation, WI), as described by Pinho et al., (2012). Primers ITS5 (WHITE et al., 1990) and LR6 (VILGALYS & HESTER, 1990) were used to amplify the rRNA-ITS region (containing internal transcribed spacer 1, 5.8S ribosomal RNA and internal transcribed spacer 2) and the D1/D2 domain of large of the subunit ribosomal rRNA gene.

Polymerase chain reactions (PCR) were prepared with 12.5 µl of Dream Taq™ PCR

Master Mix 2X; 1 µl of each forward and reverse primer synthesized by Invitrogen (Carlsbad, CA); 8.5 µl of nuclease-free water and 2 µl of genomic DNA (25 ng/µl). Negative controls were performed at each reaction, using sterile water instead of DNA.

The amplifications were performed in the C1000™ Thermal Cycler (BIO-RAD) with initial denaturation at 95 °C, for 300 s, followed by 30 cycles of denaturation, at 95 °C, for 60 s, primer annealing at 49°C, for 120 s, primer extension at 72 °C, for 120 s, and a final extension at 72 °C, for 600 s.

To confirm the amplification, PCR products were examined after conventional electrophoresis at 80 V, for 40 min, in 0.8 % agarose (w/v) gel in 1X Tris-acetate-EDTA (TAE) buffer. Then, the gels were stained with GelRed™ and visualized under UV light for the assessment of the amplicon size. The PCR product was purified and sequenced by Macrogen Inc., South Korea (<http://www.macrogen.com>).

Consensus nucleotide sequences were assembled using the FinchTV v.1.4.0 (Geospiza Inc.) software system, and the D1/D2 domain was separated from the ITS region before conducting phylogeny. Then, the consensus sequences were compared with the sequences deposited in Genbank, using the Blast (Basic Local Alignment Search Tool) for the initial identification of isolates.

The MEGA v. 7 software system (KUMAR et al., 2016) employing the MUSCLE® algorithm (EDGAR, 2004) was used for the alignment of the consensus sequences with the sequences deposited in databases. The best nucleotide substitution model for the D1/D2 domain was determined by the Akaike Information Criterion (AIC), using MrMODELTEST 2 (POSADA & BUCKLEY, 2004).

Bayesian inference (BI) analyses of the D1/D2 region were conducted in MrBayes on XSEDE v.3.2.6 (RONQUIST et al., 2012) at the CIPRES Science Gateway V. 3.3 (MILLER et al., 2015), employing the Markov Chain Monte Carlo (MCMC) method. Four MCMC chains were run simultaneously, starting from random trees for 10,000,000 generations. The trees were sampled every 1,000 generations. The first 2,500 trees out of the trees generated were discarded as the burn-in phase, and the posterior probabilities were determined from the remaining trees (RANNALA & YANG, 1996). The analysis was finished when the average standard deviation was less than 0.01. The generated trees were viewed in FigTree v. 1.4.3 (RAMBAUT, 2019) and edited with the use of graphics programs.

### 2.3 Morphological studies

Representative isolates identified in the phylogenetic analyses were used for morphological characterization. After 7 days on potato dextrose agar (PDA) (4g/L of Potato extract, 20 g/L of Dextrose, 15 g/L of Agar) and Yeast Mold (YM) Agar (3g/L of Yeast Extract, 3g/L of Malt Extract, 10g/L of Dextrose, 5g/L of Peptone, 20g/L of Agar) at 25 °C, the colony diameter was measured using a caliper rule. Microscopic preparations were mounted in lactoglycerol and photographs were taken with an Olympus BX53 microscope equipped with a digital camera, Olympus Q-Color5™. At least 30 measurements of the relevant morphological characteristics, *i.e.*, hyphae and arthroconidia, were performed using the Olympus cellSens Dimension 1.9 software system.

#### 2.4 Pathogenicity tests

Three types of healthy tomato fruits (Sweet Grape, Italian tomato and Long-self Life tomato) were selected at the same stage of maturity to be used in the fungal pathogenicity test. Ripe fruits were inoculated with the representative isolates by mechanical injury. For artificial inoculation, the isolates *G. candidum* (COAD 2969), *G. phurueaensis* (COAD 2970), *G. citri-aurantii* (COAD 2971), *Geotrichum* sp. 1 (COAD 2974), *Geotrichum* sp. 2 (COAD 2972), *Geotrichum* sp. 2 (COAD 2973), *G. vulgare* (COAD 2975), *H. burtonii* (CDA 1969), *S. vini* (CDA 2041), *M. tetrasperma* (CDA 2097), *H. khmerensis* (CDA 2446), *S. crataegensis* (CDA 2455), *T. coremiiforme* (CDA 2459), and *Z. meyeriae* (CDA 2463) were grown on PDA plates, for 7 days, at 25 °C. The CDA code means DNA extraction code, laboratory code. First, the fruits were hand washed in running tap water and neutral detergent. Then, they were disinfested by immersion in sodium hypochlorite solution (1% NaClO) for 2 minutes, rinsed in tap water and air-dried. The fungi were inoculated into hosts with sterile toothpicks, up to the depth of 2 centimeters. Each fruit was individually placed in a transparent plastic pot. Cotton pieces wetted with sterile distilled water were placed inside each plastic pot, previously pierced with a 0.70 x 25 mm needle, to maintain high humidity levels for 48 h post-inoculation. Then, the cotton pieces were removed, and the fruits were kept at the same temperature until evaluation was conducted for the presence or absence of symptoms of sour rot (incidence). As control, sterile toothpicks without fungal mycelium were deposited at the same depth on tomato fruits.

The pathogenicity of the isolate *Geotrichum* sp. 1 was also tested on potato tuber, which is the original host. Potato tubers were hand washed in running tap water to superficially remove soil. Then, they were disinfested by immersion in sodium hypochlorite solution (1% NaClO) for 10 minutes, rinsed in tap water and air-dried. The inoculation method used in the

pathogenicity test on tomato fruits was also used on potato. The potato tubers were placed in a plastic box, at 25 °C, with cotton pieces wetted with sterile distilled water to maintain high humidity levels, for 48 h post-inoculation. The plastic box was kept in the dark until evaluation for the presence or absence of symptoms of sour rot (incidence). The control treatment consisted of potato tubers wounded with a sterile toothpick without fungal mycelium.

The pathogenicity of the isolate *Geotrichum* sp. 2 was also tested on red mombin fruit, which is the original host. Ripe red mombin fruits were hand washed in running tap water and with neutral detergent. Then, they were dipped in 70% alcohol, for 2 min, and disinfested by immersion in sodium hypochlorite solution (1% NaClO), for 5 minutes, rinsed in sterile water 3 times and air-dried. The inoculation method used in the pathogenicity test on tomato fruits was also used on red mombin. The red mombin fruits were placed individually in a transparent plastic pot, at 25 °C, with cotton pieces wetted with sterile distilled water to maintain high humidity levels for 48 h post-inoculation. The cotton pieces were removed, and the fruits were kept at the same temperature until evaluation was conducted for the presence or absence of symptoms of sour rot (incidence). The control treatment consisted of red mombin fruits inoculated with a sterile toothpick without fungal mycelium.

To investigate if lower temperatures affected the development of the disease, the isolates were incubated at different temperatures. The isolates *M. tetrasperma* (CDA 2097), *G. candidum* (COAD 2969), *G. phurueaensis* (COAD 2970), *Geotrichum* sp. 1 (COAD 2974), *Geotrichum* sp. 2 (COAD 2972) and *G. vulgare* (COAD 2975) were used to perform the pathogenicity test at refrigeration temperature (8 °C). The isolates *G. candidum* (COAD 2969) and *Geotrichum* sp. 2 (COAD 2972) were used for the temperature of 19 °C, and only the species *G. vulgare* (COAD 2975) was used, for the temperature of 22 °C. The same cultivars of tomatoes, the same sanitized conditions and inoculation method used in the pathogenicity test at 25 °C were used to conduct pathogenicity at lower temperatures.

All experiments were conducted in a completely randomized design, with four replications per treatment (isolate), and one fruit per replicate. Each test was repeated 3 times.

### **3 Results**

#### **3.1 Symptomatology and fungal isolation**

The symptoms observed on the fruits of tomatoes and red mombins, in chayote and arracacha roots were postharvest rot with abundant growth of whitish mycelium on the surface

of the tissues, in addition to soaking and ethyl odor release (Fig. 1). A total of 300 isolates were obtained from these crops: 40 on *Solanum lycopersicum*, 27 on *Fragaria × ananassa*, 24 on *Solanum tuberosum*, 24 on *Spondias purpurea*, 17 on *Eugenia uniflora*, 16 on *Persea americana*, 11 on *Citrus × sinensis*, 10 on *Citrus reticulata*, 9 on *Malpighia emarginata*, 8 on *Sechium edule*, 8 on *Daucus carota* subsp. *sativus*, 8 on *Citrus × latifolia*, 8 on *Litchi chinensis*, 6 on *Petroselinum crispum*, 6 on *Citrus reticulata*, 6 on *Terminalia catappa* 5 on *Solanum grilo*, 5 on *Musa*, 5 on *Eriobotrya japonica*, 5 on *Garcinia mangostana*, , 5 on *Allium cepa*, 4 on *Lactuca sativa*, 4 on *Allium schoenoprasum*, 4 on *Citrus × limonia*, 3 on *Psidium guajava*, 3 on *Syzygium jambos*, 2 on *Beta vulgaris*, 2 on *Solanum melongena*, 2 on *Arracacia xanthorrhiza*, 2 on *Annona squamosa*, 2 on *Manihot esculenta*, 2 on *Butia capitata*, 2 on *Syagrus oleracea*, 2 on *Eugenia stipitate*, 1 on *Cucumis anguria*, 1 on *Thevetia peruviana*, 1 on *Brassica rapa pekinensis*, 1 on *Dioscorea trifida*, 1 on *Prunus cerasus*, 1 on *Bactris gasipaes*, 1 on *Averrhoa carambola*, 1 on *Mangifera indica*, 1 on *Syzygium cumini* and 1 on *Dypsis madagascariensis*. 1 on *Psidium myrtoides*, 1 on *Psidium firmum* and 1 on *Psidium cattleyanum*.

### 3.2 PCR amplification, sequencing and phylogeny

Two hundred out of the 300 isolates obtained were purified to achieve monosporic culture, DNA extraction and sequencing. A total of 45 sequences with low quality were excluded from the analyses and 165 high-quality sequences were used for phylogenetic analyses and deposited in GenBank sequence database (Table 1). High-quality sequences were compared with sequences in database, such as GenBank, using BLAST. The phylogenetic trees for each genus were generated separately, using the D1/D2 domain of large subunit ribosomal rRNA gene, which has approximately 500 nucleotides.

The alignment using only sequences of the *Geotrichum* contained 194 taxa, including outgroup and consisted of 596 base pairs, including gaps.

The phylogenetic analyses showed thirteen clades containing isolates identified as different species (Fig. 2). One clade contains all isolates of *G. candidum* with Bayesian Posterior Probabilities (BPP) with the value of 0.88. The second clade contains the strain of *Geotrichum* sp. 1 with BPP of 0.96. The third clade contains all isolates of *G. vulgare*. A fourth clade contains strains of *G. phurueaensis* (with BPP of 1). The fifth clade contains strain of *Geotrichum* sp. 2 (with BPP of 1). The sixth clade consists of isolates of *G. europaerum* (with BPP of 0.97). The seventh clade consists of isolates of *Galactomyces geotrichum* (with BPP of 1). The eighth clade consists of isolates of *G. citri-aurantii* (with BPP of 1). The ninth clade

contains strains of *Gal. ressi*. The tenth clade contains strains of *Dipodascus carabidarum* (with BPP of 1). The eleventh clade consists of isolates of *D. histeridarum* (with BPP of 1). The twelfth clade contains strains of *D. fermentans* (with BPP of 1). The thirteenth clade consists of isolates of *D. cucujoidarum* (with BPP of 1). Several clades in this phylogenetic tree contain *Geotrichum* isolates from different localities and hosts.

In phylogenetic analyses for the genus *Geotrichum*, three isolates did not cluster with any known species, probably representing two new species: *Geotrichum* sp. 1 (one isolate) and *Geotrichum* sp. 2 (two isolates) that will be proposed in accordance with the International Code of Nomenclature for algae, fungi and plants.

A total of 129 isolates grouped with *G. candidum*, 6 with *G. phurueaensis*, 2 with *G. vulgare* and 15 with *G. citri-aurantii*. Strains of *G. silvicola*, *G. bryndzae* and *Galactomyces candidum* remained in the same clade of *G. candidum* (Fig. 2).

The phylogenetic analyses of the genus *Hyphopichia* included 21 ingroup taxa and *Danielozyma ontarioensis* CBS 8502 (type strain) as outgroup taxon. The alignment of sequences consisted of 543 base pairs, including gaps. The two isolates of this study were grouped in different clades with known species. An isolate (CDA 2446) from *Eriobotrya japonica* grouped with *Hyphopichia khmerensis* (with BPP of 1) and another isolate (CDA 1969) from *Musa* grouped with *Hyphopichia burtonii* (with BPP of 0.97) (Fig. 3).

The phylogenetic analyses of the genus *Magnusiomyces* included 18 ingroup taxa with *Saccharomyces cerevisiae* NRRL Y-12632 (type strain) as outgroup taxon. The alignment of sequence dataset consisted of 505 base pairs, including gaps. In the phylogenetic tree, one isolate CDA 2097 from *Litchi chinensis* was identified as *Magnusiomyces tetrasperma* (with BPP of 0.95) (Fig. 4).

The alignment of the genus *Saccharomycopsis* comprises 36 strains, including the outgroup taxon *Wickerhamiella wanderwaltii* CBS 5524 (type strain), and the dataset consisted of 576 base pairs, including gaps. In the phylogenetic tree, four isolates were identified as known species. In a clade, two isolates (CDA 2455 and CDA 2460) from *Litchi chinensis* and one isolate (CDA 2458) from *Spondias purpurea* were identified as *S. crataegensis* (with BPP of 1) and another, the isolate CDA 2041 from *Psidium myrtilodes*, was identified as *S. vini* (with BPP of 0.99) (Fig. 5).

The phylogenetic analysis of the genus *Trichosporon* included 18 ingroup taxa with *Haglerozyma chiarellii* CBS 11177 (type strain) as outgroup taxon, and the alignment of the dataset resulted in a total of 634 characters including gaps. In the phylogenetic tree, the isolate

CDA 2459 from *Garcinia mangostana* was identified as *T. coremiiforme* (with BPP of 0.95) (Fig. 6).

Phylogenetic inference for the genus *Zygoascus* included 16 ingroup taxa with *Wickerhamiella wanderwaltii* CBS 5524 (type strain) as outgroup taxon and the alignment of sequences consisted of 568 base pairs, including gaps. In the phylogenetic tree, the isolate CDA 2463 from *Butia capitata* was identified as *Z. meyeri* (Fig. 7).

### 3.3 Taxonomy

All isolates previously identified as *Geotrichum* and *Magnusiomyces* showed typical *Geotrichum* morphology, such as arthroconidia formed in chains by the random fragmentation of hyphae, variable in size. Based on phylogenetic analyses of D1/D2 domain and morphological characteristics, seven *Geotrichum* species were distinguished, five of which are known taxa, and the other two are potential new species. One isolate was identified as a known species of *Magnusiomyces*.

In *Hyphopichia*, *Saccharomycopsis*, *Trichosporon* and *Zygoascus*, cells are ovoid to elongate and occur singly, in pairs or short chains. Based on phylogenetic analyses, two isolates were identified as known species of *Hyphopichia*, four isolates as known species of *Saccharomycopsis*, one isolate as known species of *Trichosporon* and one as known species of *Zygoascus*. The morphological characteristics of the species are shown in Table 1.

### 3.4 Systematic

#### 3.4.1 *Geotrichum* sp. 1 COAD 2974 (to be proposed as new species)

**Systematic position:** Fungi, Ascomycota, Saccharomycotina, Saccharomycetes, Saccharomycetidae, Saccharomycetales, Dipodascaceae.

After 7 days on Potato Dextrose Agar and Yeast Mold Agar, at 25 °C, in the dark, colonies sporulating attaining 5.5 and 5.7 cm of diameter, respectively. The mycelium abundantly formed is white, dry and powdery. The hyphae are 3.1–7.9 µm wide, with early disarticulation into cubic arthroconidia. Arthroconidia were abundantly present, hyaline with 3.2–5.1 µm wide and 4.8–22.0 µm long (Table 2).

**Material examined:** —BRAZIL. Goiás: Cristalina, isolated from potato tuber showing (rot symptoms), February 2018, A. Reis (VIC 47375, living culture COAD 2974).

**GenBank accession numbers:** MN930194

**Notes:** *Geotrichum* sp. 1 is phylogenetically close to *G. phurueaensis* and *G. europaeum* (Fig. 2), but grouped on a different clade. *Geotrichum* sp. 1 has hyphae up to 7.9  $\mu\text{m}$  while *G. europaeum* described by Hoog & Smith (2004) has hyphae size up to 8.0  $\mu\text{m}$ . *Geotrichum* sp. 1 has hypha width smaller than those of *G. phurueaensis* described by Kaewwichian et al., (2010) (3.1–7.9  $\mu\text{m}$  vs. 4.0–12.0  $\mu\text{m}$ ) and arthroconidia of *Geotrichum* sp. 1 were longer than *G. phurueaensis* (4.8–22.0  $\mu\text{m}$  long vs 4.0–5.0  $\mu\text{m}$ ) and wider (3.2–5.1  $\mu\text{m}$  wide vs. x 3.0–5.0  $\mu\text{m}$ ). The diameter of the colony in PDA of *Geotrichum* sp. 1 was bigger than those of *G. europaeum* described by Hoog & Smith (2004) (5.4 vs up to 5.0). For *Gal. geotrichum*, previously described by Butler et al., (1977), ascospore and sexual spore were measured. *Geotrichum* sp. 1 did not produce ascospores *in vitro*.

### 3.4.2 *Geotrichum* sp. 2 COAD 2973 (to be proposed as new species)

**Systematic position:** Fungi, Ascomycota, Saccharomycotina, Saccharomycetes, Saccharomycetidae, Saccharomycetales, Dipodascaceae

After 7 days on Potato Dextrose Agar and Yeast Mold Agar, at 25 °C, in the dark, colonies sporulating attained 5.4 and 4.5 cm of diameter, respectively. Mycelium abundantly formed is hyaline, dry and powdery. Hyphae is 3.1–6.0  $\mu\text{m}$  wide with early disarticulation into cubic arthroconidia. Abundant arthroconidia were observed, 3.2–5.1  $\mu\text{m}$  wide and 4.2–18.5  $\mu\text{m}$  long hyaline (Table 2).

**Material examined:** —BRAZIL. Goiás: Padre Bernardo, isolated from red mombin fruits (associated with rot symptoms), February 2018, A. Reis (VIC 47374, living culture COAD 2973).

**GenBank accession numbers:** MN930193

**Additional specimens examined:** —BRAZIL. Goiás: Padre Bernardo, isolated from red mombin fruits (associated with rot symptoms), February 2018, A. Reis VIC 47373 (COAD 2972), GenBank accession numbers MN930192.

**Notes:** *Geotrichum* sp. 2 is phylogenetically close to *G. phurueaensis* (Fig. 2), but grouped on a different clade. Additionally, *Geotrichum* sp. 2 differs for having hyphae shorter than the hyphae of *G. phurueaensis* described by Kaewwichian et al., (2010) (3.1–6.0  $\mu\text{m}$  vs. 4.0–12.0  $\mu\text{m}$ ). In addition, arthroconidia of *Geotrichum* sp. 2 were longer, (4.2–18.5  $\mu\text{m}$  long vs 4.0–5.0  $\mu\text{m}$ ) and wider (3.2–5.1  $\mu\text{m}$  wide vs x 3.0–5.0  $\mu\text{m}$ ).

**Notes:** *Geotrichum* sp. 1 and *Geotrichum* sp. 2 have the same minimum hyphae width (3.1  $\mu\text{m}$ ). However, *Geotrichum* sp. 2 differs from *Geotrichum* sp. 1 for having maximum hyphae width less than the hyphae of *Geotrichum* sp. 1 (3.1–6.0  $\mu\text{m}$  vs. 3.1–7.9  $\mu\text{m}$ ). Besides, arthroconidia of *Geotrichum* sp. 1 were longer than the arthroconidia of *Geotrichum* sp. 2 (4.8–22.0  $\mu\text{m}$  long vs 4.2–18.5  $\mu\text{m}$ ) and the width is the same for both species (3.2–5.1  $\mu\text{m}$ ). Besides the morphological differences, *Geotrichum* sp. 1 and *Geotrichum* sp. 2 are differentiated by phylogenetic analyses (Fig. 2).

### 3.5 Pathogenicity test

Fruits of tomatoes inoculated with *G. candidum* (COAD 2969) (Fig. 12), *G. phurueaensis* (COAD 2970) (Fig. 12), *G. citri-aurantii* (COAD 2971) (Fig. 13), *Geotrichum* sp. 1 (COAD 2974) (Fig. 8), *Geotrichum* sp. 2 (COAD 2972), *Geotrichum* sp. 2 (COAD 2973) (Fig. 9), *G. vulgare* (COAD 2975) (Fig. 13), *Hyphopichia burtonii* (CDA 1969) (Fig. 14), *Saccharomycopsis vini* (CDA 2041) (Fig. 17) and *S. crataegensis* (CDA 2455) (Fig. 18) showed symptoms 7 days after inoculation (DAI), at 25 °C, in all three tomatoes cultivars. The fruits started to crack at 2 DAI and formed a depression on the fruit surface covered with a layer of white mycelium. On the 7 DAI, the tomato fruits were already fully decayed. As the disease progresses in Long-shelf Life tomato cultivars, a liquid with a fetid odor was exuded. Unlike the Italian tomato and Sweet Grape cultivars that exude less liquid and with an ethyl odor.

When tomato fruits were inoculated with *Magnusiomyces testrasperma* (CDA 2097) (Fig. 16), *Trichosporon coremiiforme* (CDA 2459) (Fig.19) and *Zygoascus meyeriae* (CDA 2463) (Fig. 20) and incubated at 25 °C, symptoms were observed in the cultivar Long-shelf Life tomato and Sweet Grape, while on tomato fruits inoculated with *H. khmerensis* (CDA 2446) (Fig.14), the symptoms were observed on the Long-shelf Life and Italian tomato cultivars.

The *Geotrichum* sp. 1 isolate tested (COAD 2974) was pathogenic to potato tubers, its original host, at 25 °C. The inoculated tubers developed potato sour rot symptoms with dark rot of the tissue. No sour rot symptoms were observed on potato tubers of the control treatment (Fig.8).

The *Geotrichum* sp. 2 isolate (COAD 2973) was pathogenic to red mombin fruits, its original host, at 25 °C. The inoculated fruits developed sour rot symptoms with rot of the

tissue and formation of a layer of white mycelium, and at 5 DAI, red mombin fruits were already fully decayed. No sour rot symptoms were observed on red mombin fruits of the control treatment (Fig. 9).

Symptoms of sour rot were also observed when the tomato fruits were inoculated with *G. candidum* (COAD 2969) and *Geotrichum* sp. 2 (COAD 2972), kept at 19 °C (Fig. 21), and *G. vulgare* (COAD 2975), kept at 22 °C (Fig. 21). At 19 °C, the tomatoes were decayed 9 to 10 DAI.

At the temperature of 8 °C, no fungal development was observed at 32 DAI on the three tomatoes cultivars inoculated with the isolates *M. testrasperma* (CDA 2097), *G. phurueaensis* (COAD 2970), *G. candidum* (COAD 2969), *Geotrichum* sp. 2 (COAD 2972), *Geotrichum* sp. 1 (COAD 2974) and *G. vulgare* (COAD 2975). Tomatoes from the Sweet grape cultivar became wrinkled due to water loss, and the Long-shelf Life tomato and Italian tomato cultivars lost some of their colors. (Fig. 23 and 24). No symptoms were observed on tomato fruits used as control, at any of the temperatures tested.

#### 4 Discussion

This work provides new information employing molecular phylogenies, morphological comparisons and pathogenicity tests of *Geotrichum* species and other Saccharomycetidae genera associated with sour rot on fruits and vegetables in Brazil. Our phylogenetic studies suggest that there is no evidence for geographic patterns and host preferences among the *Geotrichum*-like isolates obtained. Among the species of *Geotrichum* identified, two unknown *Geotrichum* species have been described herein and will be proposed as new species. Besides *Geotrichum* species, five other genera were identified in association with fruit and vegetable sour rot in Brazil.

The taxonomy of *Geotrichum* species based on morphology presents limitations of the phenotypic characteristics to identify the genus, due to distinct species with similar morphology (BUTLER et al. 1988; HOOG & SMITH 2004; GROENEWALD et al., 2012). Thus, species recognition generally depends on comparisons based on molecular biology, as an additional tool for detecting and differentiating morphologically similar species (BICKFORD et al., 2007). Analyses of the D1/D2 domain of large subunit ribosomal rRNA gene revealed that it provided a good resolution to discriminate against the species identified here. Although the ITS region is considered the DNA barcode for fungi (SCHOCH et al.

2012), this region was not used in this phylogeny due to difficulties to align the sequences, which generated long gaps.

*Geotrichum silvicola* was described by Pimenta et al., (2005), by the analysis of the D1/D2 domain of the large subunit of the rRNA gene. The isolates were obtained from *Drosophila* flies in two Atlantic rain forest sites in Brazil and oak tasar silkworm larvae in India, but they did not include the ex-type strain of *G. candidum* (CBS 178.71) in their studies. Sulo et al., (2009), by sequence comparison of the D1/D2 domain of the large subunit of the rRNA gene, reported that *G. bryndzae* is phylogenetically close to *G. silvicola*, although the ex-type strain of *G. candidum* (CBS 178.71) was not included in their analysis, when described *G. bryndzae* isolated from Bryndza, a Slovak artisanal sheep cheese. Since the taxonomy of the genus is based on phylogenetic analyses, several taxa were transferred to other genera, and some species were synonymized (HOOG & SMITH 2004; GROENEWALD et al., 2012). Similarly, in our study, we considered that the two species, *i.e.*, *G. bryndzae* and *G. silvicola*, are synonyms of *G. candidum*, because they grouped with the type strain.

*Geotrichum* species used to be linked to the name of the two sexual morphs, *Galactomyces* and *Dipodascus* (HOOG & SMITH 2004). The International Code of Nomenclature for algae, fungi and plants established that pleomorphic fungi should have only one name (KNAPP et al., 2011; MCNEILL et al., 2012). Thus, species of *Galactomyces* were replaced by *Geotrichum*, the oldest name available for this phylogenetic group.

Type strains of *G. vulgare* (CBS 10073) and *Gal. pseudocandidum* (CBS 626.83) were grouped in the same clade. Groenewald et al., (2012) by the analyses of the D1/D2 domains of the large subunit rRNA gene, internal transcribed spacer regions (ITS) and partial actin gene, reported in their studies that these two species are conspecific, differing only by two nucleotide substitutions in the D1/D2 domain. Wuczowski et al., (2006), by the analysis of the D1/D2 domain of the large subunit rRNA gene, described *G. vulgare* from soil in Austria and from a drain in a Turkish factory of soft drinks. However, the authors did not include the type strain of *Gal. pseudocandidum* (CBS 626.83) in their analyses. Thus, the isolates that were previously identified as belonging to these two species are the same species, *i.e.*, *G. vulgare*.

*Geotrichum* has been reported as the only genus causing sour rot. However, our studies add to the evidence that some yeast genus, such as *Hyphopichia*, *Magnusiomyces*, *Saccharomycopsis*, *Trichosporon* and *Zygoascus*, are able to cause sour rot on fruit and vegetables in Brazil.

In our study, we found *H. burtonii* (CDA 1969) associated with sour rot on *Musa* fruit in Massaranduba, SC. *Hyphopichia burtonii* have already been found on other substrates and have even been used as biocontrol agents, but never as a postharvest pathogen for bananas or others fruits (CÓRDOVA et al., 2018, GUO et al., 2014; VIRGILI et al., 2012, NALLY et al., 2012, 2013, 2015).

We report *H. khmerensis* (CDA 2446) associated with postharvest disease on *Eriobotrya japonica* fruit in Gama, DF, unlike what was reported by Nagatsuka et al., (2005), in which this species has been isolated from fermented and dry salted shrimp and sewage, but never associated with sour rot on *E. japonica*.

Diverse yeast species have been isolated from insects and their related substrates, including rotten wood, frass and galleries (REN et al., 2015; RIBEIRO et al., 2017). For example, *H. paragotoi* was isolated from the gut of insect larvae and decayed wood (REN et al., 2015). Moreover, *H. buzzinii* was isolated from rotting wood collected in an Atlantic rain forest in Brazil, and from *Clidema* sp., in French Guiana (RIBEIRO et al., 2017).

In this study, we found *M. tetrasperma* (CDA 2097) associated with postharvest disease on *Litchi chinensis*, in São Gortado, MG. However, there are no reports of *Magnusiomyces* causing postharvest rot on fruits. In the literature, there are reports of *Magnusiomyces capitatus* associated with disease in immunocompromised patients, including for example those with neutropenia, catheterization, cytotoxic chemotherapy, hematological malignancies, and chronic lung disease (BREJOVÁ et al., 2019; D'ASSUMPCAO et al., 2018; SHAH et al., 2017; TANUSKOVA et al., 2017). Besides, there are reports of *Magnusiomyces ingens* being used in the synthesis of gold nanoparticles (QU et al., 2018).

In this study, *Saccharomycopsis crataegensis* (CDA 2455) was found associated with postharvest disease on *Litchi chinensis* in Brasília, DF, *S. crataegensis* (CDA 2458) on *Spondias purpurea* in Nova Veneza, GO and *S. crataegensis* (CDA 2460) on *Litchi chinensis* in Viçosa, MG. *S. crataegensis* had previously been reported as part of the microflora of Riesling grapes grown in the Niagara peninsula, in Ontario, Canada (CHAMBERLAIN et al., 1997).

*Saccharomycopsis vini* (CDA 2041) from this study was found associated with postharvest disease on *Psidium myrtilloides* in Pelotas, RS. Barata et al., (2008) also isolated *S. vini*, but from grapes damaged by honeydew. Guerzoni et al., (1987) in a study on the analysis of the yeast flora associated with grape sour rot, found *S. vini* and *S. crataegensis* with greater density and frequency on the diseased grapes in Italy.

Although we have detected *Saccharomycopsis* species associated with postharvest sour rot on fruit, the literature reports some species found in traditional fermented beverages, such as *S. fibuligera*. Thus, these fungi have high potential of application in the fermentation industry, due to their strong amylolytic activity, secretion of amylases, acid protease and  $\beta$ -glucosidase (LIMTONG et al., 2002; CHI et al., 2009; KAYACAN et al., 2019). Bal et al., (2014) identified *S. fibuligera* in nuruk, the Korean starter culture, used to make various types of Korean alcoholic beverages, including takju, cheongju, and soju. *Saccharomycopsis fibuligera* and *S. fermentans* have been isolated from various spontaneous fermentations. Therefore, they have a long tradition to cope with artificial brewing environments (CARROLL et al., 2017; HUANG et al., 2019). *Saccharomycopsis* can also be used in the fermentation process of Daqu, a mixed culture starter, used for Chinese liquor and vinegar production (LI et al., 2017).

Besides its use in fermentative processes, there are reports of *Saccharomycopsis* with mycoparasitism activity. It can, for example, penetrate and kill other fungi (KAYACAN et al., 2019; LACHANCE et al., 2000, 2012). The phenomenon of yeast predation was demonstrated for *S. javanensis* (LACHANCE & PANG, 1997).

In this study, we found *Trichosporon coremiiforme* (CDA 2459) associated with postharvest disease on *Garcinia mangostana* in Belem, PA. However, there are reports concerning infections for some *Trichosporon* species, especially in immunocompromised patients with leukemia (RAMÍREZ & MONCADA, 2020) and liver and kidney transplant patients (SAH et al., 2019), which cause a disease called trichosporonosis (OZKAYA-PARLAKAY et al., 2016), but not as postharvest pathogen for *Garcinia mangostana*. Ozkaya-Parlakay et al., (2016) reported trichosporonosis caused by *T. asahii* on a patient with Ewing sarcoma.

In this study, the species *Zygoascus meyeræ* (CDA 2463) was associated with postharvest on *Butia capitata*, in Porto Belo, SC. Barata et al., (2008) reported a different species, *Z. hellenicus*, recovered from grapes damaged by honeydew and sour rot in different vintages and vineyards in Portugal. Besides *Trichosporon* species, there are reports concerning infections for some *Zygoascus* species, especially in immunocompromised patients (IOAKIMIDOU et al., 2011). *Zygoascus hellenicus* has been reported as a human pathogen causing fungemia in an allogeneic stem cell transplant recipient (BRANDT et al., 2004) and bovine mastitis (SMITH et al., 2005). *Zygoascus meyeræ* has been reported as a causer of respiratory infection in a child with acute myeloid leukemia (IOAKIMIDOU et al.,

2011).

The pathogenicity tests were convincing for all three cultivars of tomato fruits when inoculated with *G. candidum* (COAD 2969), *G. phurueaensis* (COAD 2970), *G. citri-aurantii* (COAD 2971), *Geotrichum* sp. 1 (COAD 2974), *Geotrichum* sp. 2 (COAD 2972), *Geotrichum* sp. 2 (COAD 2973), *G. vulgare* (COAD 2975), *Hyphopichia burtonii* (CDA 1969) *Saccharomyces vini* (CDA 2041) and *S. crataegensis* (CDA 2455) and incubated at 25°C, since all tomato fruits showed the same symptoms observed in the host in the field. Even after repeating pathogenicity tests three times, at 25 °C, the Italian tomato cultivar presented no mycelial growth of yeast *M. tetrasperma* (CDA 2097), *T. coremiiforme* (CDA 2459) or *Z. meyeriae* (CDA 2463). The results suggest that the Italian tomato cultivar used in these tests has some resistance to these three yeast species. Sweet grape tomato cultivar has some resistance against *H. khmerensis* (CDA 2446), since no growth was observed even after the experiment was repeated three times, at 25 °C.

The fungi *G. candidum* (COAD 2969) and *Geotrichum* sp. 2 (COAD 2972) developed on tomato fruits even at 19 °C, while *G. vulgare* (COAD 2975) developed at 22 °C. At these temperatures, it took the tomato fruits 3 more days to deteriorate, compared to the fruits stored at 25 °C. *Geotrichum* species grow well at the temperature of 25 °C (Sutton et al., 1998). The results indicate that temperatures other than the optimum growth temperature affect the growth of these fungi.

Most microorganisms have a satisfactory temperature range to develop, usually 20-30 °C. The knowledge of the optimal growth temperature is fundamental for the use of temperature as a control method (ZAUZA et al., 2016). Growth inhibition at refrigeration temperature (8 °C) of species *M. tetrasperma* (CDA 2097), *G. phurueaensis* (COAD 2970), *Geotrichum* sp. 2 (COAD 2972) and *G. vulgare* (COAD 2975) suggests that low temperature can be used as a strategy to slow down the growth of these pathogens.

In summary, this work demonstrates that other *Geotrichum* species besides the species *Geotrichum candidum* are associated with sour rot on fruits and vegetables in Brazil and describes two new *Geotrichum* species. In addition, five yeast genera are reported for the first time as causal agents of sour rot on fruits and vegetables. This study contributes with several taxonomic novelties for the knowledge about the diversity of fungi associated with sour rot in Brazil.

Studies on the possible formation of biofilm should be conducted to assist in the management of the disease in food processing sites, especially in minimally processed food.

The identification of these fungi is the basic strategy to prevent and control the disease, by evaluating the effects of different sanitizers, for example, considering that they may perform different activities, depending on the population of the organisms that cause the disease.

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## CONCLUSÕES

Este estudo contribui para o conhecimento da diversidade de fungos e leveduras associados à doença podridão azeda em frutas e hortaliças no Brasil, e conseqüentemente, na escolha de estratégias para o controle desta doença.

Além da espécie *Geotrichum candidum*, existem outras espécies de *Geotrichum* e algumas de leveduras tais como *G. phurueaensis*, *G. vulgare*, *G. citri-aurantii*, *H. burtonii*, *H. khmerensis*, *S. crataegensis*, *S. vini*, *M. tetrasperma*, *T. coremiiforme* e *Z. meyeriae*, associadas à podridão azeda em frutas e hortaliças no Brasil.

Duas possíveis espécies novas de *Geotrichum* foram identificadas e são capazes de causar podridão azeda em frutos de seriguela e tubérculos de batata no Brasil. Essas serão futuramente propostas de acordo com o Código Internacional de Nomenclatura para Algas, Fungos e Plantas.

Table 1: GenBank accession numbers of DNA sequences of *Geotrichum*, *Hyphopichia*, *Magnusiomyces*, *Saccharomycopsis*, *Trichosporon* and *Zygoascus* species, used in the phylogenetic analyses.

Genbank	Species	Strain code	Locality	Host	Scientific name
MN930032	<i>Geotrichum candidum</i>	CDA 1911	Padre Bernardo - GO	Beet - root	<i>Beta vulgaris</i>
MN930033	<i>Geotrichum citri-aurantii</i>	CDA 1933	Águas Claras - DF	Pokan - fruit	<i>Citrus reticulata</i>
MN930034	<i>Geotrichum candidum</i>	CDA 1952	Ceilândia - DF	Chayote - fruit	<i>Sechium edule</i>
MN930035	<i>Geotrichum candidum</i>	CDA 1953	Goianápolis - GO	Surinam cherry - fruit	<i>Eugenia uniflora</i>
MN930036	<i>Geotrichum candidum</i>	CDA 1954	Chã Grande - PE	Eggplant - fruit	<i>Solanum melongena</i>
MN930037	<i>Geotrichum candidum</i>	CDA 1955	Brazlândia - DF	Strawberry - fruit	<i>Fragaria</i> × <i>ananassa</i>
MN930038	<i>Geotrichum candidum</i>	CDA 1956	Cristalina - GO	Onion - bulb	<i>Allium cepa</i>
MN930039	<i>Geotrichum candidum</i>	CDA 1957	Corumbá - GO	Cherry - fruit	<i>Eugenia uniflora</i>
MN930040	<i>Geotrichum candidum</i>	CDA 1958	Pelotas - RS	Loquat - fruit	<i>Eriobotrya japonica</i>
MN930041	<i>Geotrichum citri-aurantii</i>	CDA 1959	Tijucas - SC	Lemon cravo - fruit	<i>Citrus</i> × <i>limonia</i>
MN930042	<i>Geotrichum candidum</i>	CDA 1961	Perdizes - MG	Potato - Seeding	<i>Solanum tuberosum</i>
MN930043	<i>Geotrichum candidum</i>	CDA 1964	Brazlândia - DF	Scarlet eggplant	<i>Solanum gilo</i>
MN930044	<i>Geotrichum candidum</i>	CDA 1965	Matinhos - PR	Royal Palm - fruit	<i>Roystonea oleracea</i>
MN930045	<i>Geotrichum candidum</i>	CDA 1967	Ceilândia - DF	Scarlet eggplant	<i>Solanum gilo</i>
MN930046	<i>Geotrichum candidum</i>	CDA 1968	Ceilândia - DF	Chayote - fruit	<i>Sechium edule</i>
MN930047	<i>Hyphopichia burtonii</i>	CDA 1969	Massaranduba - SC	Plantain - fruit	<i>Musa</i> × <i>paradisiaca</i>
MN930048	<i>Geotrichum candidum</i>	CDA 1970	Goiânia - GO	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930049	<i>Geotrichum candidum</i>	CDA 1972	Brazlândia - DF	Strawberry - fruit	<i>Fragaria</i> × <i>ananassa</i>
MN930050	<i>Geotrichum candidum</i>	CDA 1976	Cristalina - GO	Potato - Tuber	<i>Solanum tuberosum</i>
MN930051	<i>Geotrichum citri-aurantii</i>	CDA 1977	Tijucas - SC	Sour Orange - fruit	<i>Citrus</i> × <i>aurantium</i>
MN930052	<i>Geotrichum citri-aurantii</i>	CDA 1981	Taguatinga Sul - DF	Pokan - fruit	<i>Citrus reticulata</i>
MN930053	<i>Geotrichum candidum</i>	CDA 1992	Mogi das Cruzes - SP	Avocado - fruit	<i>Persea americana</i>
MN930054	<i>Geotrichum candidum</i>	CDA 1994	Tijucas - SC	Rose apple - fruit	<i>Syzygium jambos</i>
MN930055	<i>Geotrichum candidum</i>	CDA 1999	Vargem Bonita - DF	Red mombin - fruit	<i>Spondias purpurea</i>
MN930056	<i>Geotrichum candidum</i>	CDA 2000	Luziânia - GO	Avocado - fruit	<i>Persea americana</i>

Genbank	Species	Strain code	Locality	Host	Scientific name
MN930057	<i>Geotrichum candidum</i>	CDA 2001	Tijucas - SC	Sweetsop - fruit	<i>Annona squamosa</i>
MN930058	<i>Geotrichum candidum</i>	CDA 2002	Guarapuava - PR	Potato - Tuber	<i>Solanum tuberosum</i>
MN930059	<i>Geotrichum candidum</i>	CDA 2003	Samambaia - DF	Avocado - fruit	<i>Persea americana</i>
MN930060	<i>Geotrichum candidum</i>	CDA 2005	Pirenópolis - GO	Catole palm - fruit	<i>Syagrus oleracea</i>
MN930061	<i>Geotrichum candidum</i>	CDA 2006	Belém - PA	Mangosteen - fruit	<i>Garcinia mangostana</i>
MN930062	<i>Geotrichum candidum</i>	CDA 2008	Rancho queimado - SC	Strawberry - fruit	<i>Fragaria</i> × <i>ananassa</i>
MN930063	<i>Geotrichum candidum</i>	CDA 2010	Tijucas - SC	Almond - fruit	<i>Terminalia catappa</i>
MN930064	<i>Geotrichum candidum</i>	CDA 2011	Vargem Bonita - DF	Red mombin- fruit	<i>Spondias purpurea</i>
MN930065	<i>Geotrichum candidum</i>	CDA 2012	Vargem Bonita - DF	Red mombin - fruit	<i>Spondias purpurea</i>
MN930066	<i>Geotrichum candidum</i>	CDA 2013	Vargem Bonita - DF	Red mombin- fruit	<i>Spondias purpurea</i>
MN930067	<i>Geotrichum candidum</i>	CDA 2014	Viçosa - MG	Araçá Boi - fruit	<i>Eugenia stipitata</i>
MN930068	<i>Geotrichum candidum</i>	CDA 2015	Viçosa - MG	Yams - tuber	<i>Dioscorea trifida</i>
MN930069	<i>Geotrichum candidum</i>	CDA 2016	Ceilândia - DF	Chayote - fruit	<i>Sechium edule</i>
MN930070	<i>Geotrichum candidum</i>	CDA 2017	Padre Bernardo - GO	Gerkin - fruit	<i>Cucumis anguria</i>
MN930071	<i>Geotrichum candidum</i>	CDA 2018	Corumbá - GO	Strawberry - fruit	<i>Fragaria</i> × <i>ananassa</i>
MN930072	<i>Geotrichum candidum</i>	CDA 2020	Goiânia - GO	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930073	<i>Geotrichum candidum</i>	CDA 2021	Tijucas - SC	Sweetsop - fruit	<i>Annona squamosa</i>
MN930074	<i>Geotrichum candidum</i>	CDA 2023	Brasília de Minas - MG	Java plum - fruit	<i>Syzygium cumini</i>
MN930075	<i>Geotrichum candidum</i>	CDA 2026	Gama - DF	Avocado - fruit	<i>Persea americana</i>
MN930076	<i>Geotrichum candidum</i>	CDA 2038	Camucim São Félix - PE	Scarlet eggplant	<i>Solanum gilo</i>
MN930077	<i>Geotrichum candidum</i>	CDA 2039	Taguatinga Sul - DF	Loquat - fruit	<i>Eriobotrya japonica</i>
MN930078	<i>Saccharomycopsis vini</i>	CDA 2041	Pelotas - RS	Araçá Roxo - fruit	<i>Psidium myrtoides</i>
MN930079	<i>Geotrichum candidum</i>	CDA 2042	Pelotas - RS	Surinam cherry - fruit	<i>Eugenia uniflora</i>
MN930080	<i>Geotrichum candidum</i>	CDA 2043	Gama - DF	Table tomato - fruit	<i>Solanum lycopersicum</i>
MN930081	<i>Geotrichum candidum</i>	CDA 2044	Rio Grande - RS	Table tomato - fruit	<i>Solanum lycopersicum</i>
MN930082	<i>Geotrichum candidum</i>	CDA 2045	Brasília - DF	Carrot - root	<i>Daucus carota subsp. sativus</i>
MN930083	<i>Geotrichum candidum</i>	CDA 2046	Brazlândia - DF	Strawberry - fruit	<i>Fragaria</i> × <i>ananassa</i>

Genbank	Species	Strain code	Locality	Host	Scientific name
MN930084	<i>Geotrichum candidum</i>	CDA 2047	Padre Bernardo - GO	Avocado - fruit	<i>Persea americana</i>
MN930085	<i>Geotrichum candidum</i>	CDA 2049	Brazlândia - DF	Strawberry - fruit	Fragaria × ananassa
MN930086	<i>Geotrichum candidum</i>	CDA 2050	Ceilândia - DF	Chayote - fruit	<i>Sechium edule</i>
MN930087	<i>Geotrichum citri-aurantii</i>	CDA 2051	Taguatinga Sul - DF	Chapéu de Napoleão - fruit	<i>Thevetia peruviana</i>
MN930088	<i>Geotrichum candidum</i>	CDA 2055	Cristalina - GO	Onion - bulbo	<i>Allium cepa</i>
MN930089	<i>Geotrichum candidum</i>	CDA 2060	Itapema - SC	Almond - fruit	<i>Terminalia catappa</i>
MN930090	<i>Geotrichum candidum</i>	CDA 2063	Planaltina - DF	Processed carrot	<i>Daucus carota subsp. sativus</i>
MN930091	<i>Geotrichum candidum</i>	CDA 2064	Canoinhas - SC	Potato - tuber	<i>Solanum tuberosum</i>
MN930092	<i>Geotrichum vulgare</i>	CDA 2065	Cristalina - GO	Potato - tuber	<i>Solanum tuberosum</i>
MN930093	<i>Geotrichum candidum</i>	CDA 2067	Luiz Alves - SC	Manzano Banana - fruit	<i>Musa sapientum</i>
MN930094	<i>Geotrichum candidum</i>	CDA 2069	Gama - DF	Table tomato	<i>Solanum lycopersicum</i>
MN930095	<i>Geotrichum candidum</i>	CDA 2070	Marechal Floriano - ES	Arracacha - root	<i>Arracacia xanthorrhiza</i>
MN930096	<i>Geotrichum candidum</i>	CDA 2071	Estiva - MG	Strawberry - fruit	Fragaria × ananassa
MN930097	<i>Geotrichum candidum</i>	CDA 2072	Tijucas - SC	Loquat - fruit	<i>Eriobotrya japonica</i>
MN930098	<i>Geotrichum candidum</i>	CDA 2073	Cristalina - GO	Potato - tuber	<i>Solanum tuberosum</i>
MN930099	<i>Geotrichum candidum</i>	CDA 2074	Guarapuava - PR	Potato - tuber	<i>Solanum tuberosum</i>
MN930100	<i>Geotrichum candidum</i>	CDA 2075	Guarapuava - PR	Potato - tuber	<i>Solanum tuberosum</i>
MN930101	<i>Geotrichum candidum</i>	CDA 2080	Perdizes - MG	Potato - tuber	<i>Solanum tuberosum</i>
MN930102	<i>Geotrichum candidum</i>	CDA 2085	Águas Lindas - GO	Avocado - fruit	<i>Persea americana</i>
MN930103	<i>Geotrichum candidum</i>	CDA 2087	Farroupilha - RS	Strawberry - fruit	Fragaria × ananassa
MN930104	<i>Geotrichum candidum</i>	CDA 2088	Gama - DF	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930105	<i>Geotrichum candidum</i>	CDA 2089	Cristalina - GO	Carrot - root	<i>Daucus carota subsp. sativus</i>
MN930106	<i>Geotrichum candidum</i>	CDA 2090	Padre Bernardo - GO	Avocado - fruit	<i>Persea americana</i>
MN930107	<i>Geotrichum phurueaensis</i>	CDA 2092	Altamira - PA	Barbados cherry- fruit	<i>Malpighia emarginata</i>
MN930108	<i>Geotrichum candidum</i>	CDA 2093	Gama - DF	Onion - bulbo	<i>Allium cepa</i>
MN930109	<i>Geotrichum candidum</i>	CDA 2096	Mauá da Serra - PR	Tangerine fruit	<i>Citrus reticulata</i>
MN930110	<i>Magnusiomyces tetrasperma</i>	CDA 2097	São Gotardo - MG	Lychee - fruit	<i>Litchi chinensis</i>

Genbank	Species	Strain code	Locality	Host	Scientific name
MN930111	<i>Geotrichum candidum</i>	CDA 2099	Luiz Alves - SC	Manzano Banana - fruit	<i>Musa sapientum</i>
MN930112	<i>Geotrichum candidum</i>	CDA 2100	Pelotas - RS	Surinam cherry - fruit	<i>Eugenia uniflora</i>
MN930114	<i>Geotrichum candidum</i>	CDA 2102	Gama - DF	Tomato – fruit	<i>Solanum lycopersicum</i>
MN930115	<i>Geotrichum candidum</i>	CDA 2103	Brazlândia - DF	Tahiti lemon - fruit	Citrus × latifolia
MN930116	<i>Geotrichum citri-aurantii</i>	CDA 2104	Holambra - SP	Sweet orange - fruit	<i>Citrus sinensis</i>
MN930117	<i>Geotrichum candidum</i>	CDA 2202	Itapema - SC	Almond - fruit	<i>Terminalia catappa</i>
MN930118	<i>Geotrichum candidum</i>	CDA 2203	Brasília de Minas - MG	Red mombin - fruit	<i>Spondias purpurea</i>
MN930119	<i>Geotrichum candidum</i>	CDA 2204	Brazlândia - DF	Curly lettuce - folha	<i>Lactuca sativa</i>
MN930120	<i>Geotrichum phurueaensis</i>	CDA 2205	Taguatinga Sul - DF	Suriname cherry - fruit	<i>Eugenia uniflora</i>
MN930121	<i>Geotrichum candidum</i>	CDA 2206	Anapólis - GO	Suriname cherry - fruit	<i>Eugenia uniflora</i>
MN930122	<i>Geotrichum candidum</i>	CDA 2207	Cristalina - GO	Carrot - root	<i>Daucus carota subsp. sativus</i>
MN930123	<i>Geotrichum candidum</i>	CDA 2208	Brazlândia - DF	Strawberry - fruit	Fragaria × ananassa
MN930124	<i>Geotrichum candidum</i>	CDA 2209	Alfredo Wagner - SC	Strawberry - fruit	Fragaria × ananassa
MN930125	<i>Geotrichum candidum</i>	CDA 2210	Tijucas - SC	Loquat - fruit	<i>Eriobotrya japonica</i>
MN930126	<i>Geotrichum candidum</i>	CDA 2211	Tijucas - SC	Cowberry- fruit	<i>Malpighia emarginata</i>
MN930127	<i>Geotrichum citri-aurantii</i>	CDA 2212	Tijucas - SC	Tahiti lemon - fruit	Citrus × latifolia
MN930128	<i>Geotrichum candidum</i>	CDA 2213	Tijucas - SC	Tahiti lemon - fruit	Citrus × latifolia
MN930129	<i>Geotrichum candidum</i>	CDA 2215	Vargem Bonita - DF	Red mombin - fruit	<i>Spondias purpurea</i>
MN930130	<i>Geotrichum candidum</i>	CDA 2216	Viçosa - MG	Lychee - fruit	<i>Litchi chinensis</i>
MN930131	<i>Geotrichum candidum</i>	CDA 2217	Itapema - SC	Almond - fruit	<i>Terminalia catappa</i>
MN930132	<i>Geotrichum candidum</i>	CDA 2218	Belém - PA	Mangosteen - fruit	<i>Garcinia mangostana</i>
MN930133	<i>Geotrichum candidum</i>	CDA 2219	Claro das Poções - MG	Chayote - fruit	<i>Sechium edule</i>
MN930134	<i>Geotrichum candidum</i>	CDA 2221	Planaltina - DF	Table tomato - fruit	<i>Solanum lycopersicum</i>
MN930135	<i>Geotrichum candidum</i>	CDA 2222	Araucária - PR	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930136	<i>Geotrichum candidum</i>	CDA 2223	Brazlândia - DF	Strawberry - fruit	Fragaria × ananassa
MN930137	<i>Geotrichum candidum</i>	CDA 2224	Luiz Alves - SC	Yellow Cavendish Banana - fruit	<i>Musa acuminata 'Dwarf Cavendish'</i>
MN930138	<i>Geotrichum candidum</i>	CDA 2225	Tijucas - SC	Avocado - fruit	<i>Persea americana</i>

<b>Genbank</b>	<b>Species</b>	<b>Strain code</b>	<b>Locality</b>	<b>Host</b>	<b>Scientific name</b>
MN930139	<i>Geotrichum candidum</i>	CDA 2227	Brasília - DF	Carrot - root	<i>Daucus carota subsp. sativus</i>
MN930140	<i>Geotrichum candidum</i>	CDA 2288	Brasília - DF	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930141	<i>Geotrichum candidum</i>	CDA 2290	Samambaia - DF	Welsh onion - pseudostem	<i>Allium schoenoprasum</i>
MN930142	<i>Geotrichum candidum</i>	CDA 2291	Samambaia - DF	Welsh onion - pseudostem	<i>Allium schoenoprasum</i>
MN930143	<i>Geotrichum candidum</i>	CDA 2292	São Gabriel - GO	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930144	<i>Geotrichum candidum</i>	CDA 2293	São Gabriel - GO	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930145	<i>Geotrichum candidum</i>	CDA 2294	São Gabriel - GO	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930146	<i>Geotrichum candidum</i>	CDA 2295	São Gabriel - GO	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930147	<i>Geotrichum phurueaensis</i>	CDA 2296	Recife - PE	Red mombin - fruit	<i>Spondias purpurea</i>
MN930148	<i>Geotrichum phurueaensis</i>	CDA 2297	Recife - PE	Red mombin - fruit	<i>Spondias purpurea</i>
MN930149	<i>Geotrichum candidum</i>	CDA 2300	Goianópolis - GO	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930150	<i>Geotrichum candidum</i>	CDA 2303	Cristalina - GO	Onion - bulbo	<i>Allium cepa</i>
MN930151	<i>Geotrichum candidum</i>	CDA 2304	Padre Bernardo - GO	Cassava - root	<i>Manihot esculenta</i>
MN930152	<i>Geotrichum candidum</i>	CDA 2305	Reserva - PR	Tomato - stem	<i>Solanum lycopersicum</i>
MN930153	<i>Geotrichum citri-aurantii</i>	CDA 2306	Cidade Estrutural - DF	Parsley - root	<i>Petroselinum crispum</i>
MN930154	<i>Geotrichum candidum</i>	CDA 2308	Cidade Estrutural - DF	Parsley - root	<i>Petroselinum crispum</i>
MN930155	<i>Geotrichum candidum</i>	CDA 2309	Cidade Estrutural - DF	Parsley - root	<i>Petroselinum crispum</i>
MN930156	<i>Geotrichum candidum</i>	CDA 2312	Samambaia - DF	Chinese cabbage	<i>Brassica rapa</i>
MN930157	<i>Geotrichum candidum</i>	CDA 2313	Guarapuava - PR	Potato - Tuber	<i>Solanum tuberosum</i>
MN930158	<i>Geotrichum candidum</i>	CDA 2420	Viçosa - MG	Scarlet eggplant	<i>Solanum gilo</i>
MN930159	<i>Geotrichum candidum</i>	CDA 2423	Belém - PA	Mangosteen - fruit	<i>Garcinia mangostana</i>
MN930160	<i>Geotrichum citri-aurantii</i>	CDA 2424	Perdizes - MG	Potato - tuber	<i>Solanum tuberosum</i>
MN930161	<i>Geotrichum candidum</i>	CDA 2425	Mucugê - BA	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930162	<i>Geotrichum candidum</i>	CDA 2426	Cristalina - GO	Carrot - root	<i>Daucus carota subsp. sativus</i>
MN930163	<i>Geotrichum candidum</i>	CDA 2427	Padre Bernardo - GO	Avocado - fruit	<i>Persea americana</i>
MN930164	<i>Geotrichum candidum</i>	CDA 2428	Cristalina - GO	Suriname cherry - fruit	<i>Eugenia uniflora</i>
MN930165	<i>Geotrichum candidum</i>	CDA 2429	Brazlândia - DF	Araçá do Cerrado - fruit	<i>Psidium firmum</i>

Genbank	Species	Strain code	Locality	Host	Scientific name
MN930166	<i>Geotrichum candidum</i>	CDA 2430	Guarapuava - PR	Potato - tuber	<i>Solanum tuberosum</i>
MN930167	<i>Geotrichum candidum</i>	CDA 2431	Guarapuava - PR	Potato - tuber	<i>Solanum tuberosum</i>
MN930168	<i>Geotrichum candidum</i>	CDA 2432	Guarapuava - PR	Potato - tuber	<i>Solanum tuberosum</i>
MN930169	<i>Geotrichum candidum</i>	CDA 2433	Guarapuava - PR	Potato - tuber	<i>Solanum tuberosum</i>
MN930170	<i>Geotrichum citri-aurantii</i>	CDA 2434	Porto Belo - SC	Sour Orange - fruit	<i>Citrus × aurantium</i>
MN930171	<i>Geotrichum citri-aurantii</i>	CDA 2437	Brazlândia - DF	Pokan - folha	<i>Citrus reticulata</i>
MN930172	<i>Geotrichum citri-aurantii</i>	CDA 2438	Águas Claras - DF	Pokan - fruit	<i>Citrus reticulata</i>
MN930173	<i>Geotrichum candidum</i>	CDA 2440	Padre Bernardo - GO	Beet -root	<i>Beta vulgaris</i>
MN930174	<i>Geotrichum citri-aurantii</i>	CDA 2442	Itaberaí - GO	Sweet orange - fruit	<i>Citrus sinensis</i>
MN930175	<i>Geotrichum citri-aurantii</i>	CDA 2443	Tijucas - SC	Tangerine - fruit	<i>Citrus reticulata</i>
MN930176	<i>Geotrichum candidum</i>	CDA 2444	Planaltina - DF	Gerkin- fruit	<i>Cucumis anguria</i>
MN930177	<i>Hyphopichia khmerensis</i>	CDA 2446	Gama - DF	Loquat - fruit	<i>Eriobotrya japonica</i>
MN930178	<i>Geotrichum candidum</i>	CDA 2447	Belém - PA	Red mombin - fruit	<i>Spondias purpurea</i>
MN930179	<i>Geotrichum phurueaensis</i>	CDA 2448	Ananindeua - PA	Cowberry- fruit	<i>Malpighia emarginata</i>
MN930180	<i>Geotrichum candidum</i>	CDA 2449	Guarapuava - PR	Potato - tuber	<i>Solanum tuberosum</i>
MN930181	<i>Geotrichum candidum</i>	CDA 2453	Serra do Salitre - MG	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930182	<i>Geotrichum candidum</i>	CDA 2454	Brasília - DF	Tomato - fruit	<i>Solanum lycopersicum</i>
MN930183	<i>Saccharomycopsis crataegensis</i>	CDA 2455	Brasília - DF	Lychee - fruit	<i>Litchi chinensis</i>
MN930184	<i>Saccharomycopsis crataegensis</i>	CDA 2458	Nova Veneza - GO	Red mombin - fruit	<i>Spondias purpurea</i>
MN930185	<i>Trichosporon coremiiforme</i>	CDA 2459	Belém - PA	Mangosteen - fruit	<i>Garcinia mangostana</i>
MN930186	<i>Saccharomycopsis crataegensis</i>	CDA 2460	Viçosa - MG	Lychee - fruit	<i>Litchi chinensis</i>
MN930187	<i>Zygoascus meyeriae</i>	CDA 2463	Porto Belo - SC	Butia - fruit	<i>Butia capitata</i>
MN930188	<i>Geotrichum candidum</i>	CDA1950	Cristalina - GO	Potato - tuber	<i>Solanum tuberosum</i>
MN930189	<i>Geotrichum candidum</i>	COAD 2969	Taguatinga Sul - DF	Almond - fruit	<i>Terminalia catappa</i>
MN930190	<i>Geotrichum phurueaensis</i>	COAD 2970	Nova Veneza - GO	Red mombin - fruit	<i>Spondias purpurea</i>

Genbank	Species	Strain code	Locality	Host	Scientific name
MN930191	<i>Geotrichum citri-aurantii</i>	COAD 2971	Perdizes - MG	Potato - tuber	<i>Solanum tuberosum</i>
MN930192	<i>Geotrichum</i> sp.	COAD 2972	Padre Bernardo - GO	Red mombin - fruit	<i>Spondias purpurea</i>
MN930193	<i>Geotrichum</i> sp.	COAD 2973	Padre Bernardo - GO	Red mombin - fruit	<i>Spondias purpurea</i>
MN930194	<i>Geotrichum</i> sp.	COAD 2974	Cidade Estrutural - DF	Potato - tuber	<i>Solanum tuberosum</i>
MN930195	<i>Geotrichum vulgare</i>	COAD 2975	Cristalina - GO	Carrot - root	<i>Daucus carota</i> subsp. <i>sativus</i>

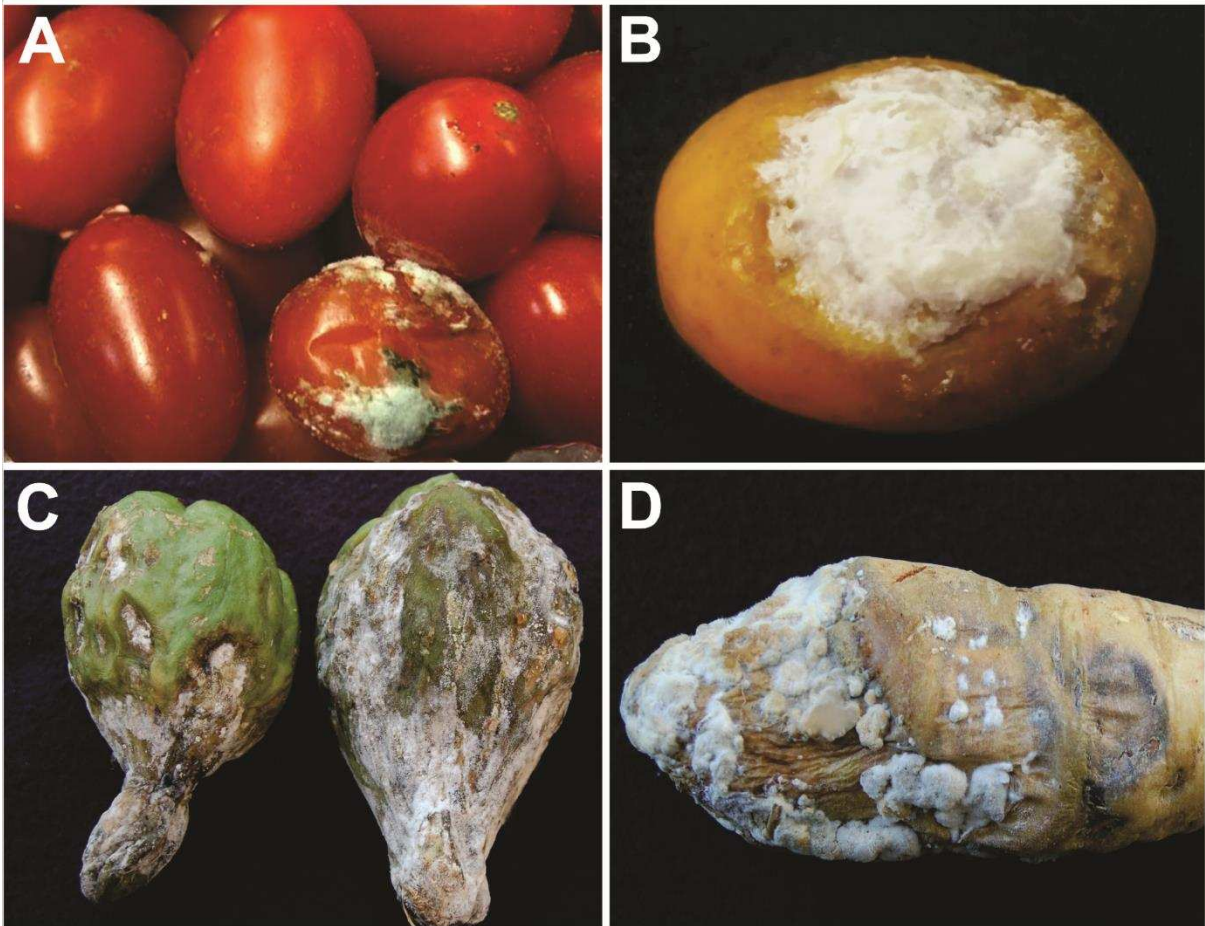
Table 2: Main morphological characteristics of *Geotrichum*, *Hyphopichia*, *Magnusiomyces*, *Saccharomycopsis*, *Trichosporon* and *Zygoascus* species.

Species	Hyphae*	Conidia*		Diameter of the colony**			Reference
		Length	Width	PDA		YM	
<i>Galactomyces citri-aurantii</i>	2.3 – 5.3	4.6 – 12.2	3.0 – 5.0	4.9	-	6.4	<b>This study</b>
<i>Geotrichum candidum</i>	2.8 – 5.6	5.8 – 14.9	2.7 – 5.7	4.3	-	5.4	<b>This study</b>
<i>Geotrichum phurueaensis</i>	2.7 – 4.8	5.6 – 23.2	4.0 – 6.3	2.9	-	3.8	<b>This study</b>
<i>Geotrichum</i> sp. 1	3.1 – 7.9	4.8 – 22.0	3.2 – 5.1	5.5	-	5.7	<b>This study</b>
<i>Geotrichum europaeum</i> <sup>T</sup>	Up to 8.0	-	-	4.1 – 5.0	-	-	Hoog & Smith, 2004
<i>Galactomyces geotrichum</i> <sup>T</sup>	-	-	-	-	-	-	Butler et al., 1977
<i>Geotrichum</i> sp. 2	3.1 – 6.0	4.2 – 18.5	3.2 – 5.1	5.4	-	4.5	<b>This study</b>
<i>Geotrichum phurueaensis</i> <sup>T</sup>	4.0 – 12.0	4.0 – 5.0	3.0 – 5.0	-	-	-	Kaewwichian et al., 2010
<i>Geotrichum vulgare</i>	2.2 – 5.1	2.9 – 11.2	2.1 – 3.6	3.2	-	6.3	<b>This study</b>
<i>Hyphopichia burtonii</i>	1.5 – 2.8	2.3 – 4.6	1.7 – 3.0	1.9***	1.7***	2.1	<b>This study</b>
<i>Hyphopichia khmerensis</i>	1.4 – 4.4	1.0 – 1.9	0.6 – 1.5	3.2***	2.8***	2.0	<b>This study</b>
<i>Magnusiomyces tetrasperma</i>	2.4 – 4.9	8.0 – 17.4	3.2 – 8.8	2.6***	2.5***	3.1	<b>This study</b>
<i>Saccharomycopsis crataegensis</i>	1.9 – 3.6	4.3 – 9.5	2.2 – 3.4	2.8***	2.5***	1.9	<b>This study</b>
<i>Saccharomycopsis vini</i>	1.8 – 3.2	4.0 – 8.1	2.1 – 3.3	4.2***	3.9***	4.0	<b>This study</b>
<i>Trichosporon coremiiforme</i>	1.5 – 4.2	2.4 – 11.5	1.9 – 3.1	2.8	-	2.7	<b>This study</b>
<i>Zygoascus meyeriae</i>	1.3 – 2.7	3.1 – 12.1	1.5 – 3.1	1.5***	1.5***	1.4	<b>This study</b>

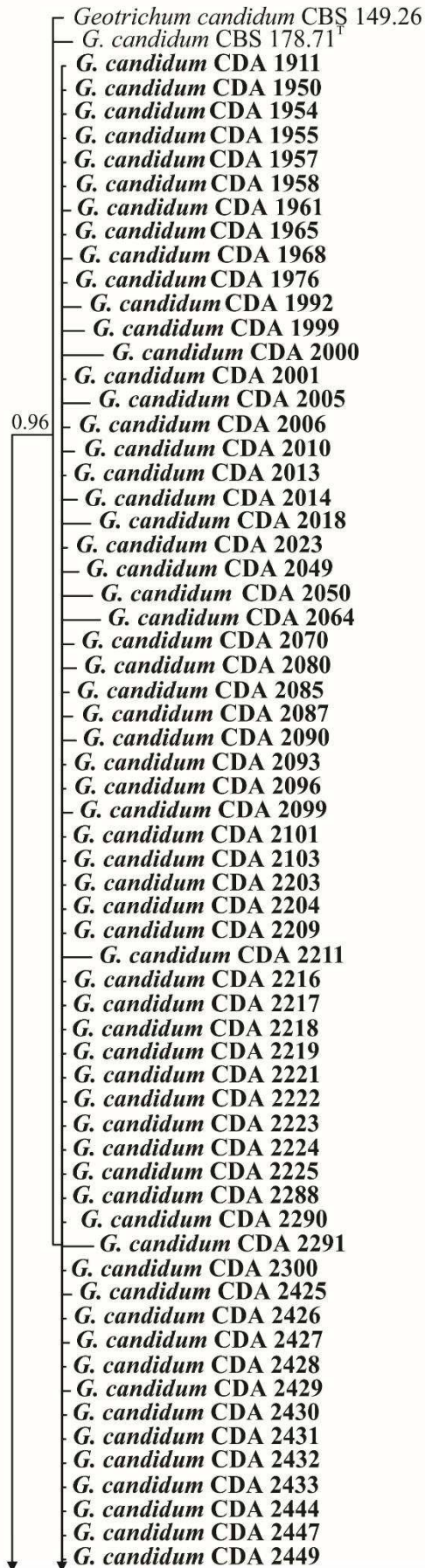
\* + in  $\mu\text{m}$ .

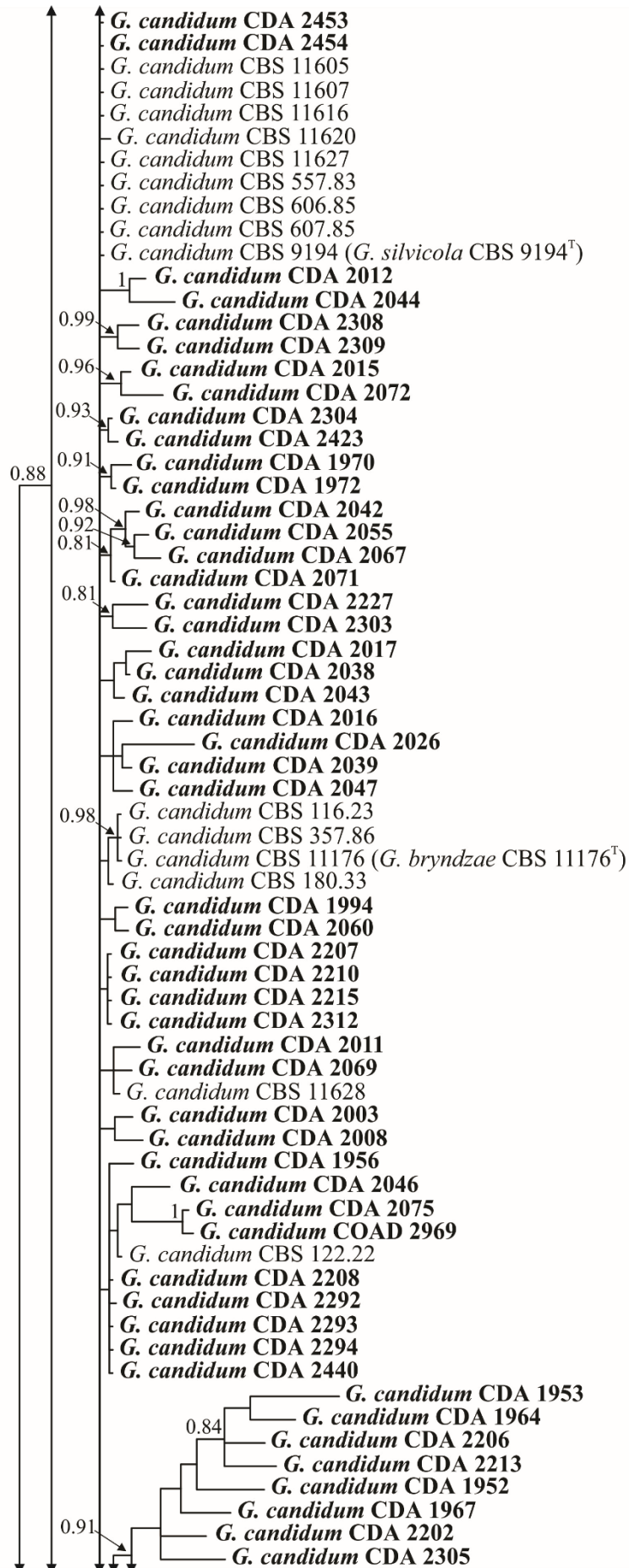
\*\* Measures in mm

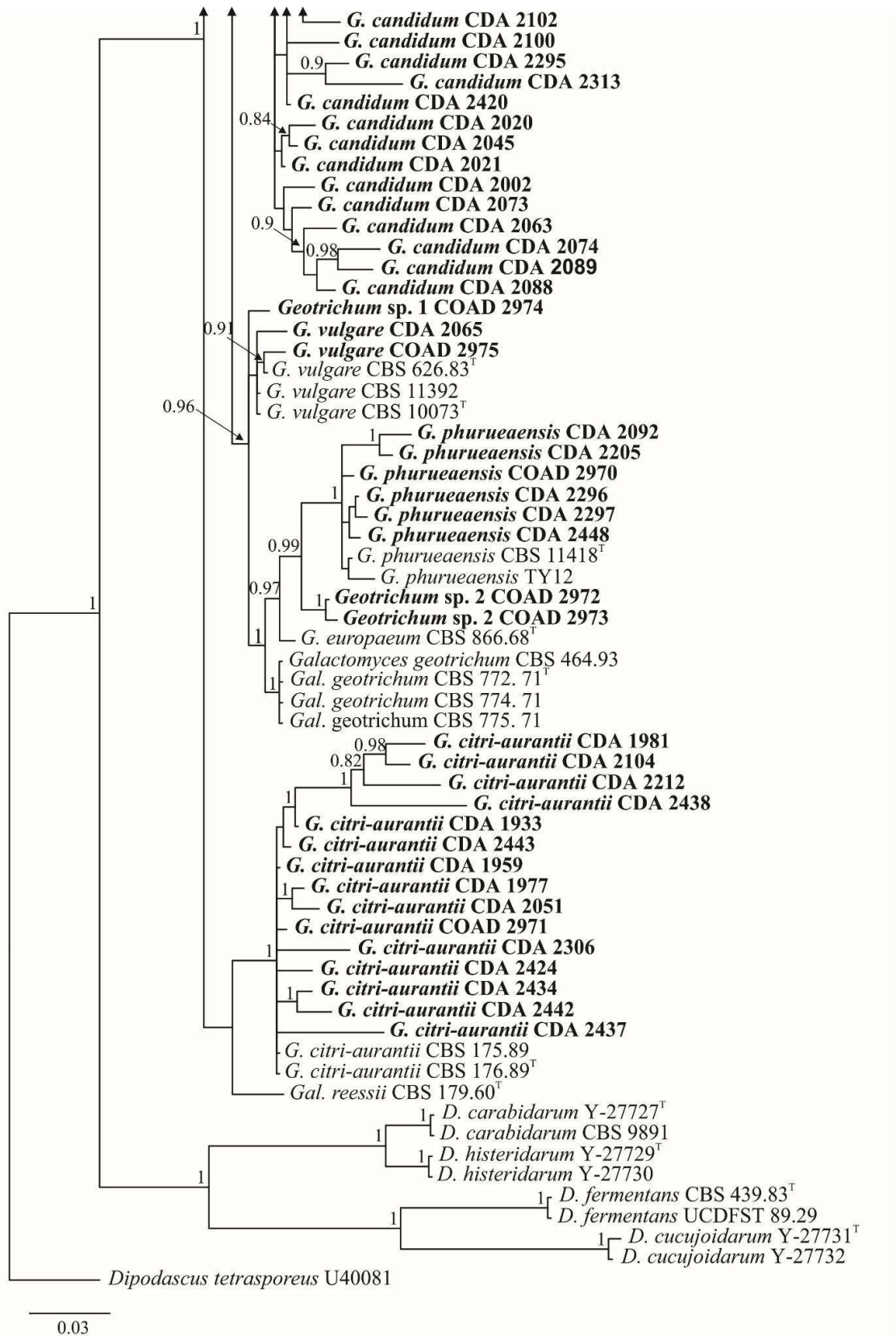
\*\*\* Measured in two dimensions due to irregular growth.



**FIGURE 1.** Symptoms of sour rot disease on different fruit and vegetable species. **a.** Tomato; **b.** Red mombin; **c.** Chayote; **d.** Arracha.

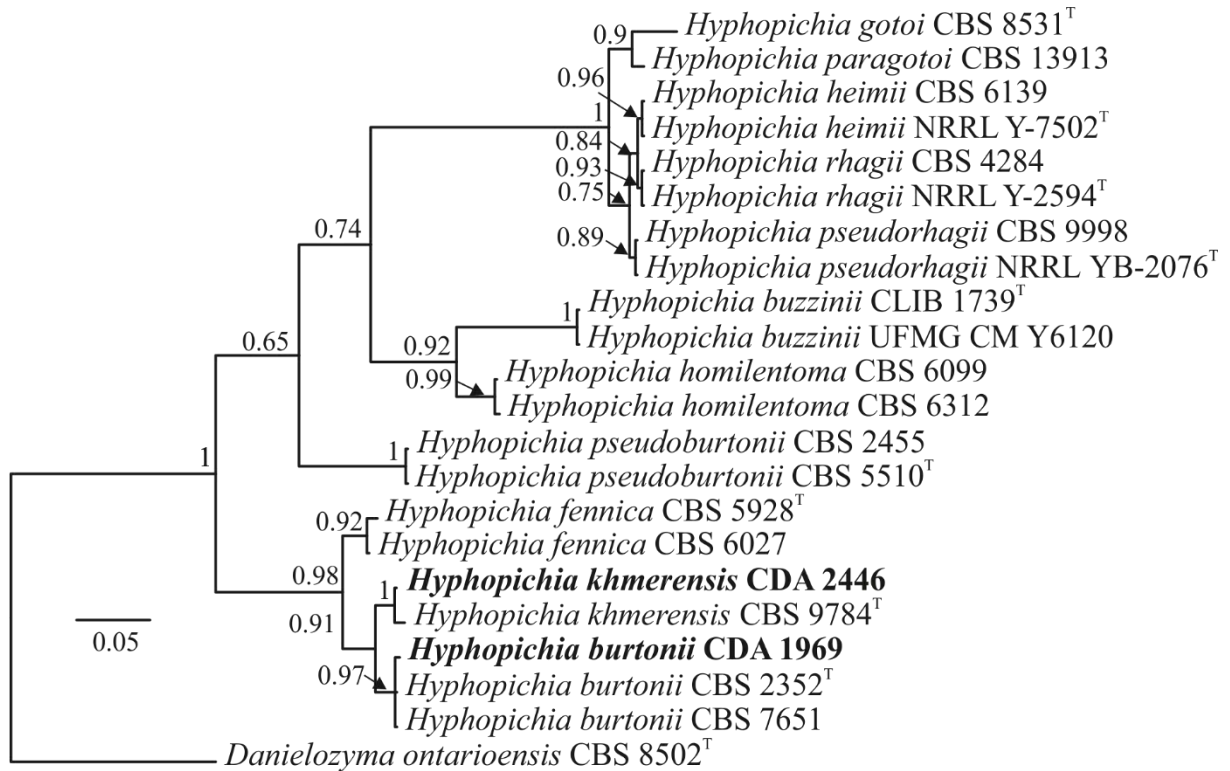




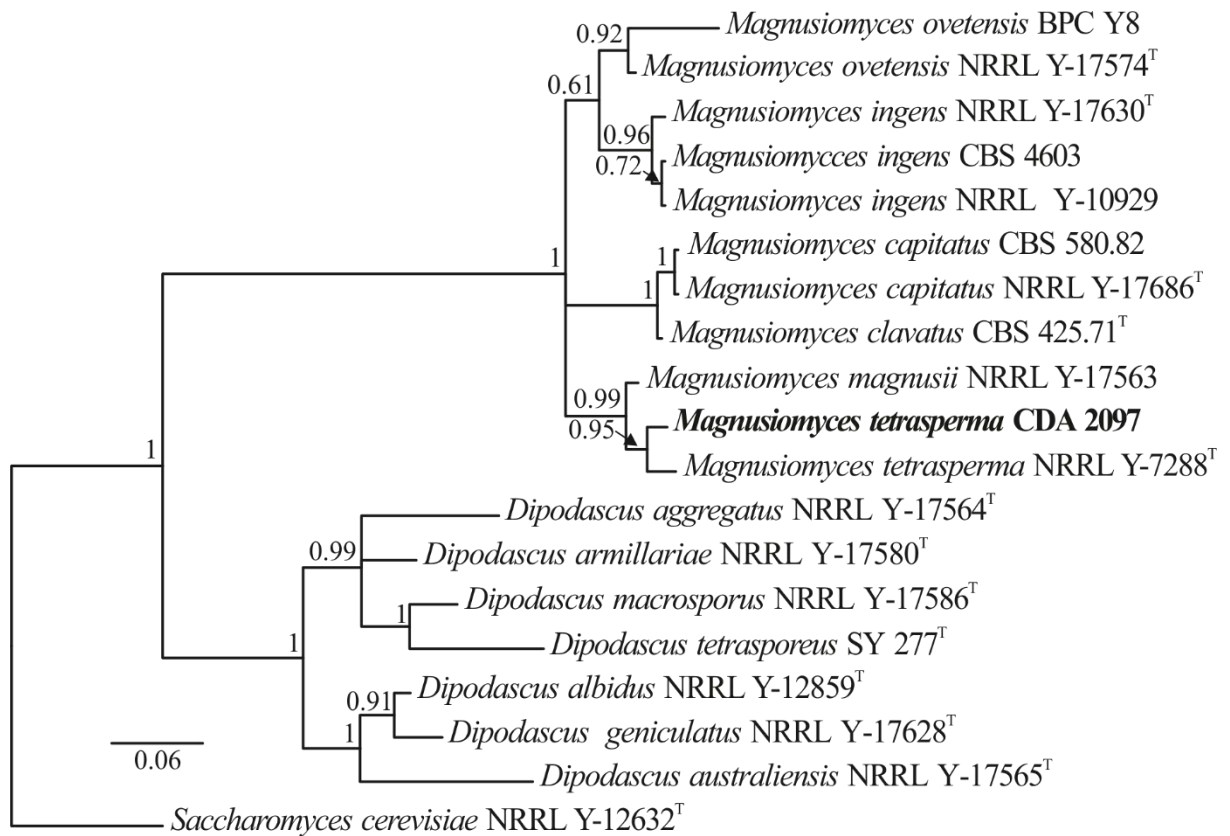


**FIGURE 2.** Phylogram of resulting trees inferred from Bayesian analysis based on nucleotide sequences of the D1/D2 region in the 26S rRNA genes for *Geotrichum* species. Bayesian posterior probabilities above 0.80 are indicated next to the nodes. The scale bar refers to the

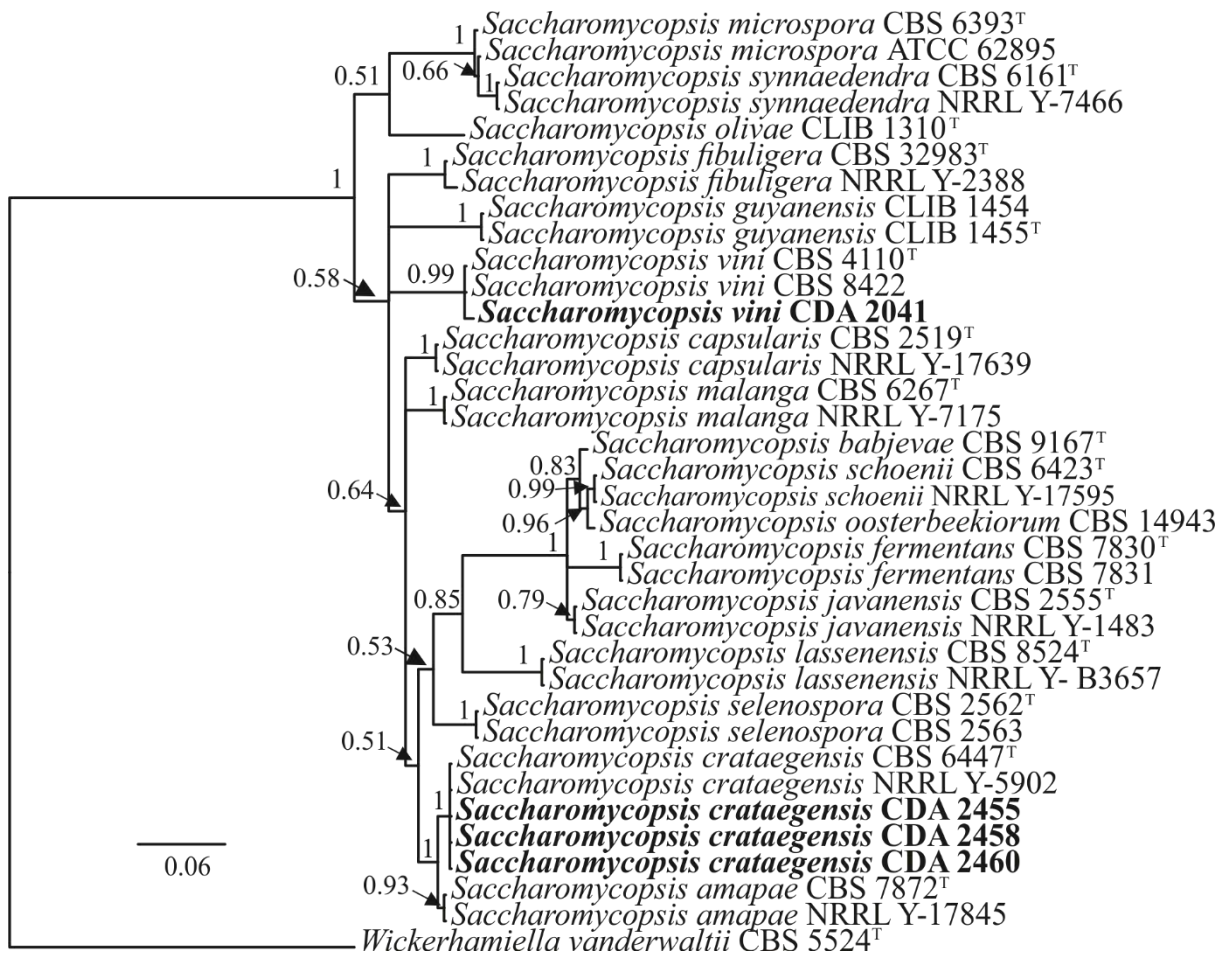
expected changes per site. The species in this study are highlighted in bold. Type strains of species are indicated after the culture collection number (T). The tree was rooted with *Dipodascus tetrasporeus* U40081.



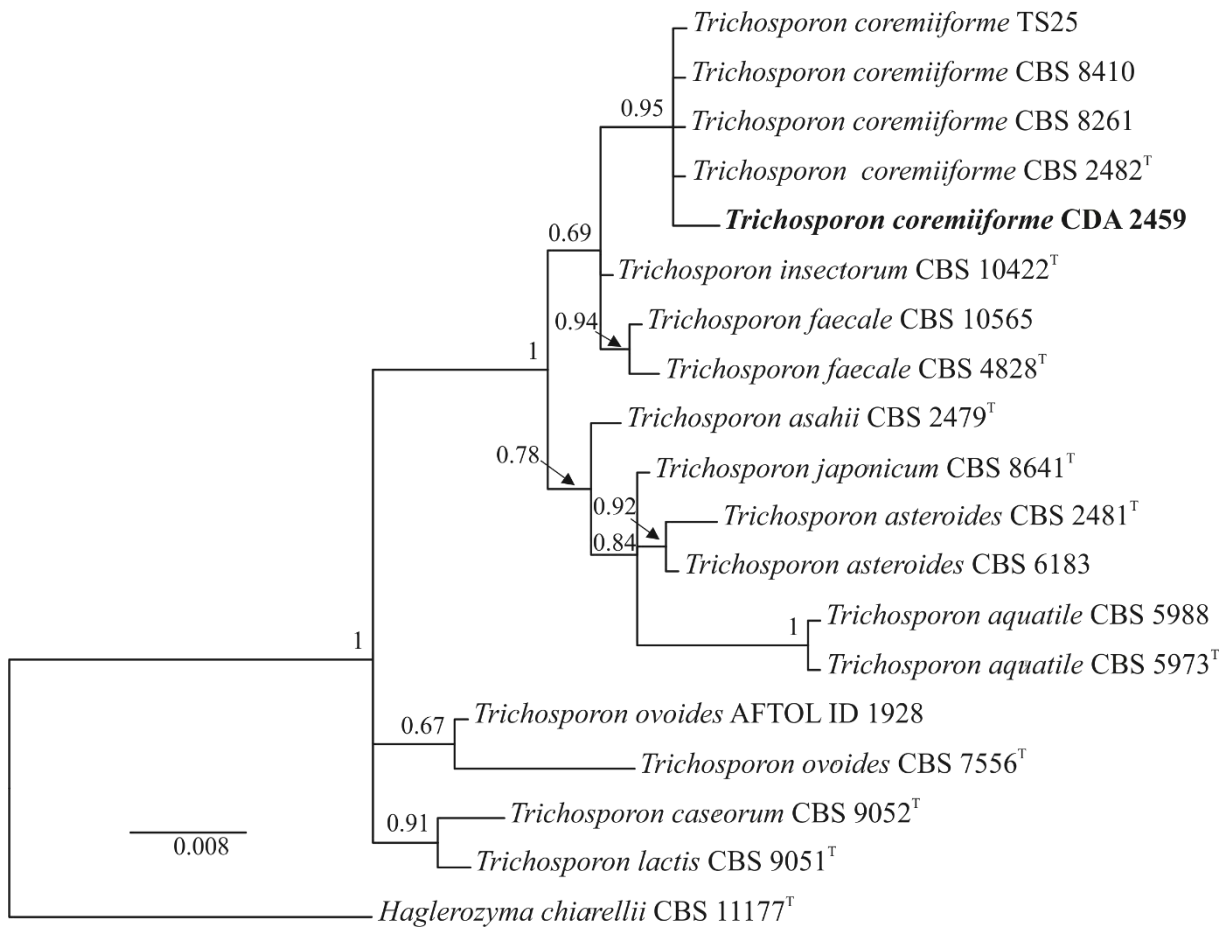
**FIGURE 3.** Phylogram of resulting trees inferred from Bayesian analysis based on nucleotide sequences of the D1/D2 region in the 26S rRNA genes for *Hyphopichia* species. Bayesian posterior probabilities are indicated next to the nodes. The scale bar refers to the expected changes per site. The species in this study are highlighted in bold. Type strains of species are indicated after the culture collection number (T). The tree was rooted with *Danielozyma ontarioensis* CBS 8502.



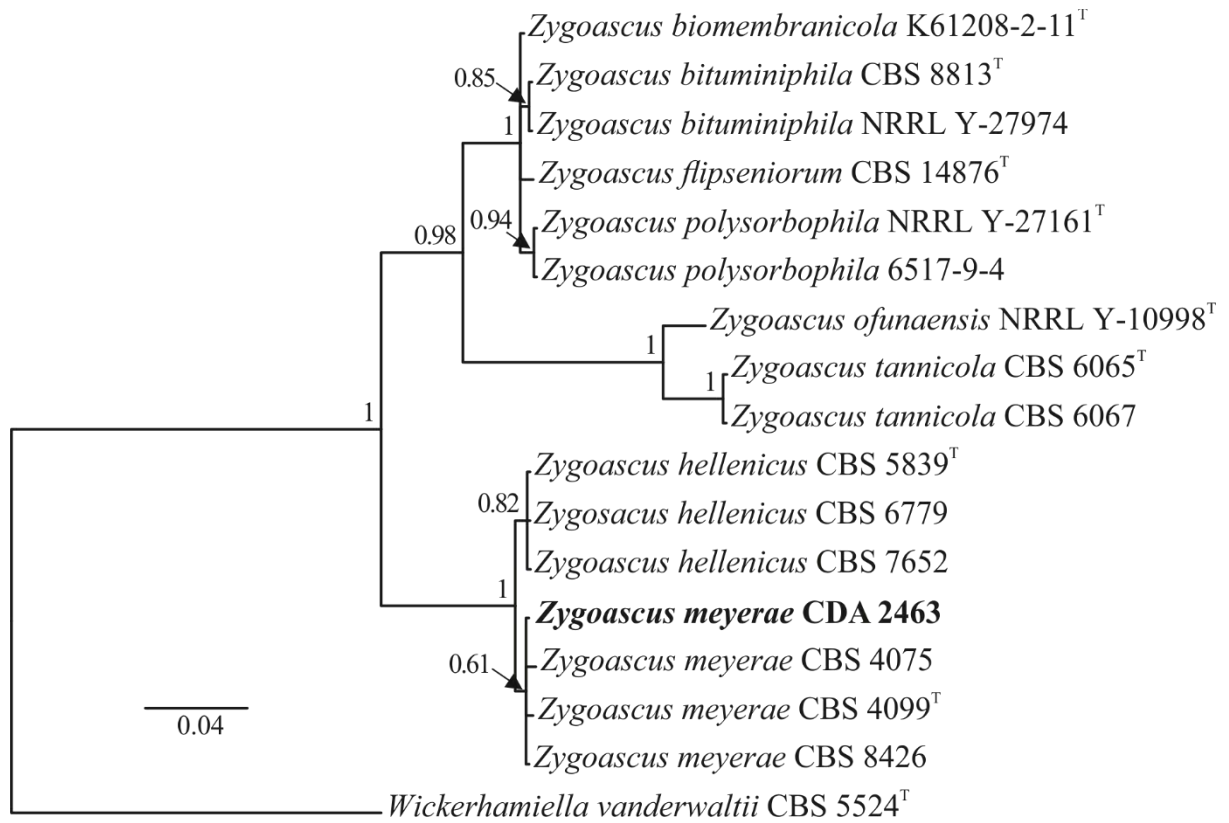
**FIGURE 4.** Phylogram of resulting trees inferred from Bayesian analysis based on nucleotide sequences of the D1/D2 region in the 26S rRNA genes for *Magnusiomyces* species. Bayesian posterior probabilities are indicated next to the nodes. The scale bar refers to the expected changes per site. The species in this study are highlighted in bold. Type strains of species are indicated after the culture collection number (T). The tree was rooted with *Saccharomyces cerevisiae* NRRL Y-12632.



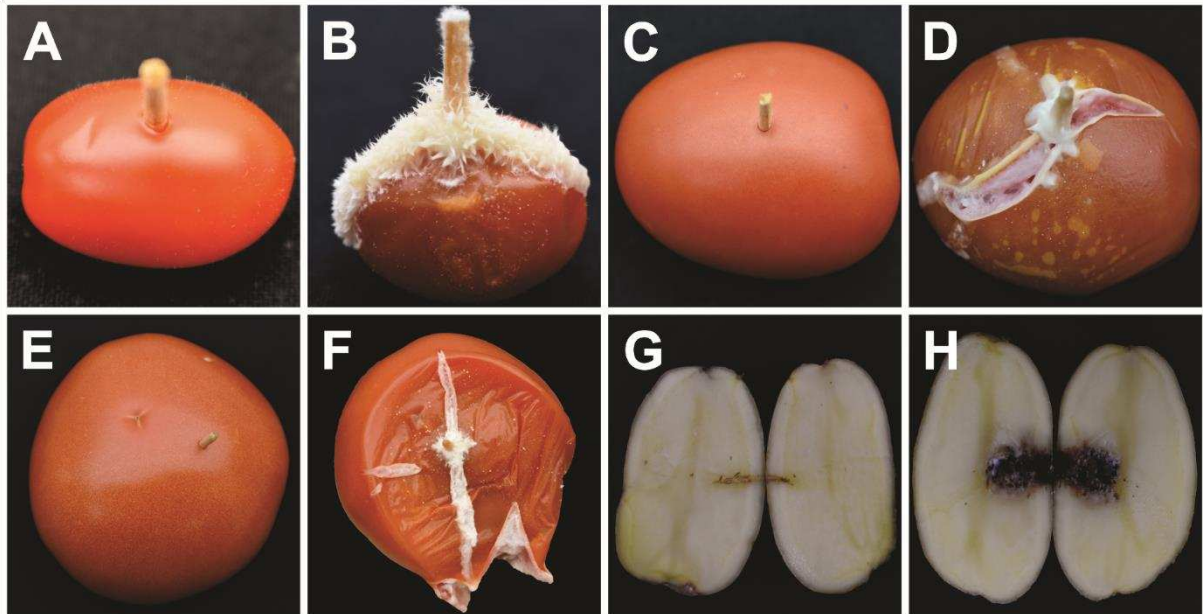
**FIGURE 5.** Phylogram of resulting trees inferred from Bayesian analysis based on nucleotide sequences of the D1/D2 region in the 26S rRNA genes for *Saccharomyces* species. Bayesian posterior probabilities are indicated next to the nodes. The scale bar refers to the expected changes per site. The species in this study are highlighted in bold. Type strains of species are indicated after the culture collection number (T). The tree was rooted with *Wickerhamiella vanderwaltii* CBS 5524.



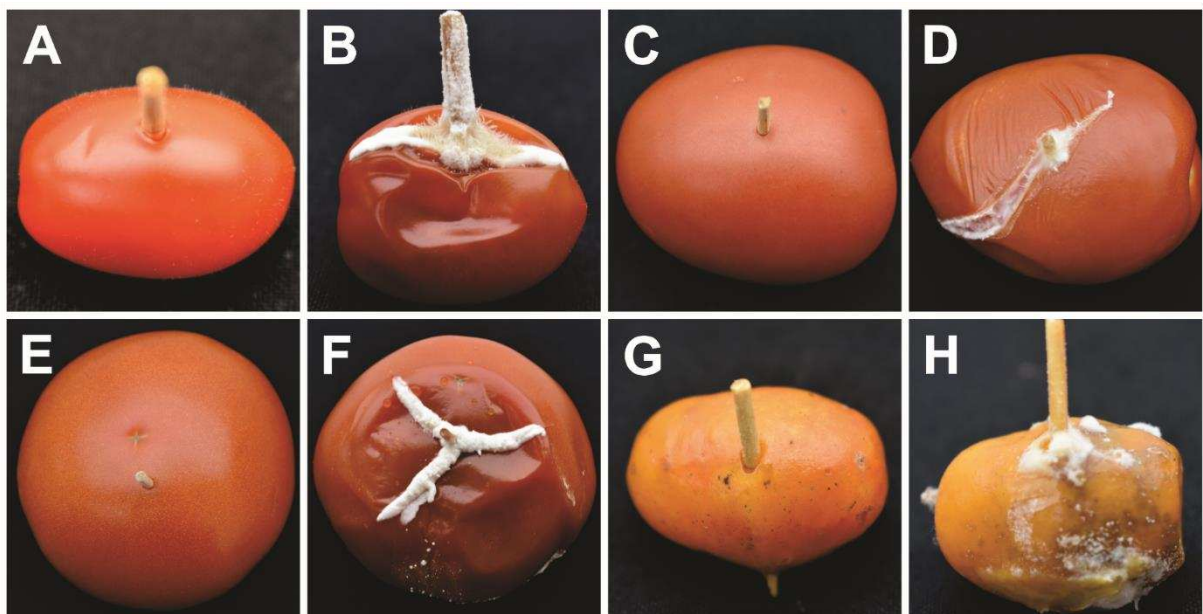
**FIGURE 6.** Phylogram of resulting trees inferred from Bayesian analysis based on nucleotide sequences of the D1/D2 region in the 26S rRNA genes for *Trichosporon* species. Bayesian posterior probabilities are indicated next to the nodes. The scale bar refers to the expected changes per site. The species in this study are highlighted in bold. Type strains of species are indicated after the culture collection number (T). The tree was rooted with *Haglerozyma chiarellii* CBS 11177.



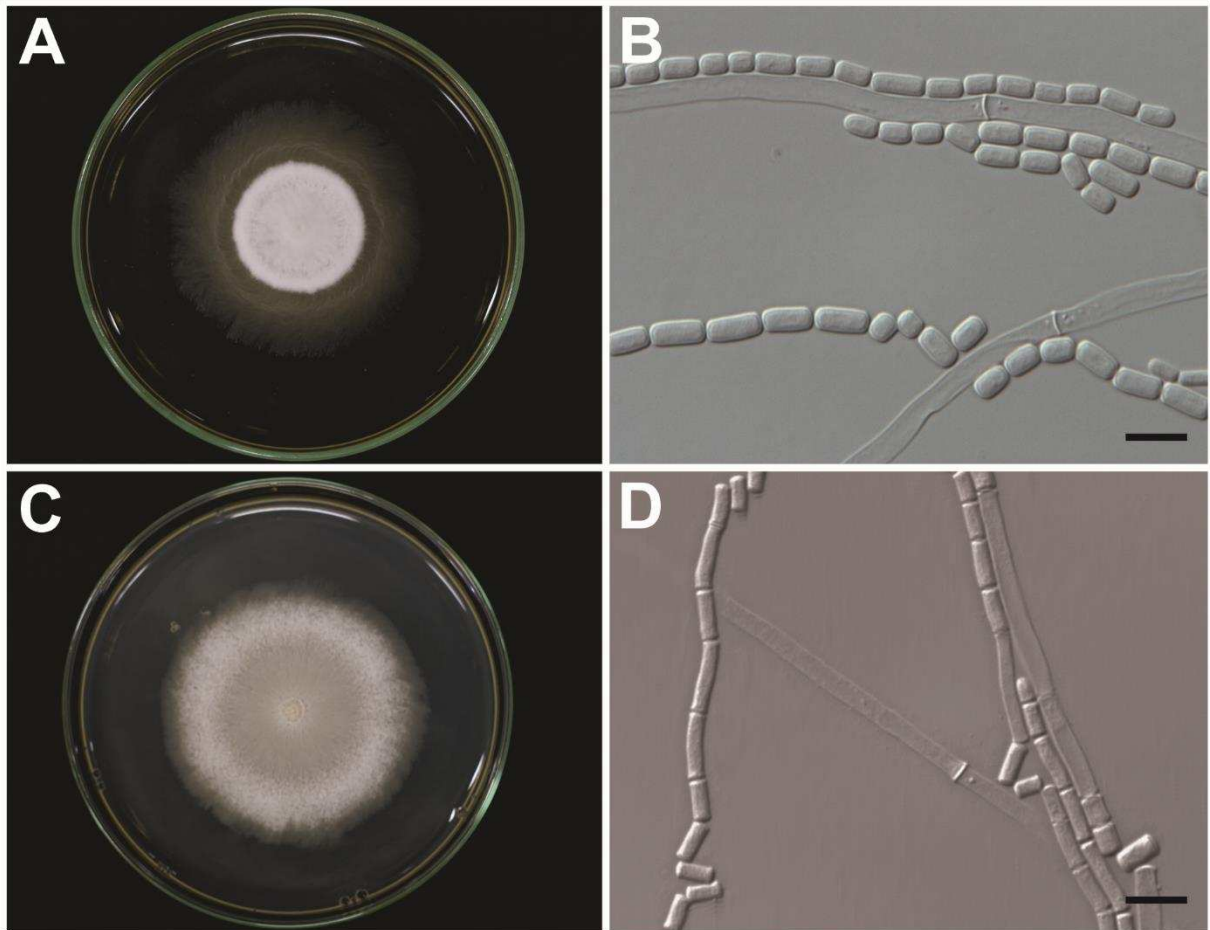
**FIGURE 7.** Phylogram of resulting trees inferred from Bayesian analysis based on nucleotide sequences of the D1/D2 region in the 26S rRNA genes for *Zygoascus* species. Bayesian posterior probabilities are indicated next to the nodes. The scale bar refers to the expected changes per site. The species in this study are highlighted in bold. Type strains of species are indicated after the culture collection number (T). The tree was rooted with *Wickerhamiella vanderwaltii* CBS 5524.



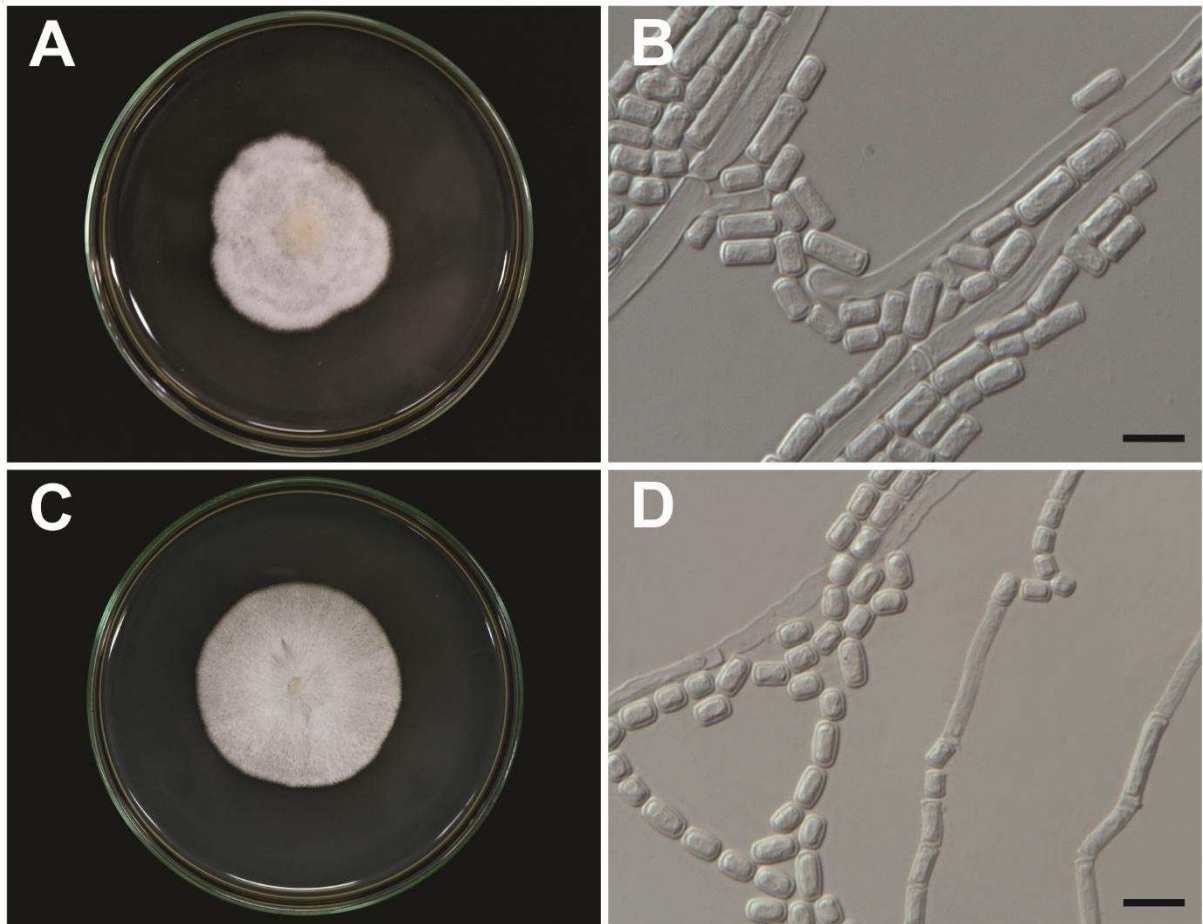
**FIGURE 8.** Pathogenicity test of *Geotrichum* sp. 1 (COAD 2974). **b.** Sour rot on sweet grape tomato; **d.** Italian tomato; **f.** Long-shelf Life tomato; **h.** Potato tubers; **a, c, e, g.** Control treatment.



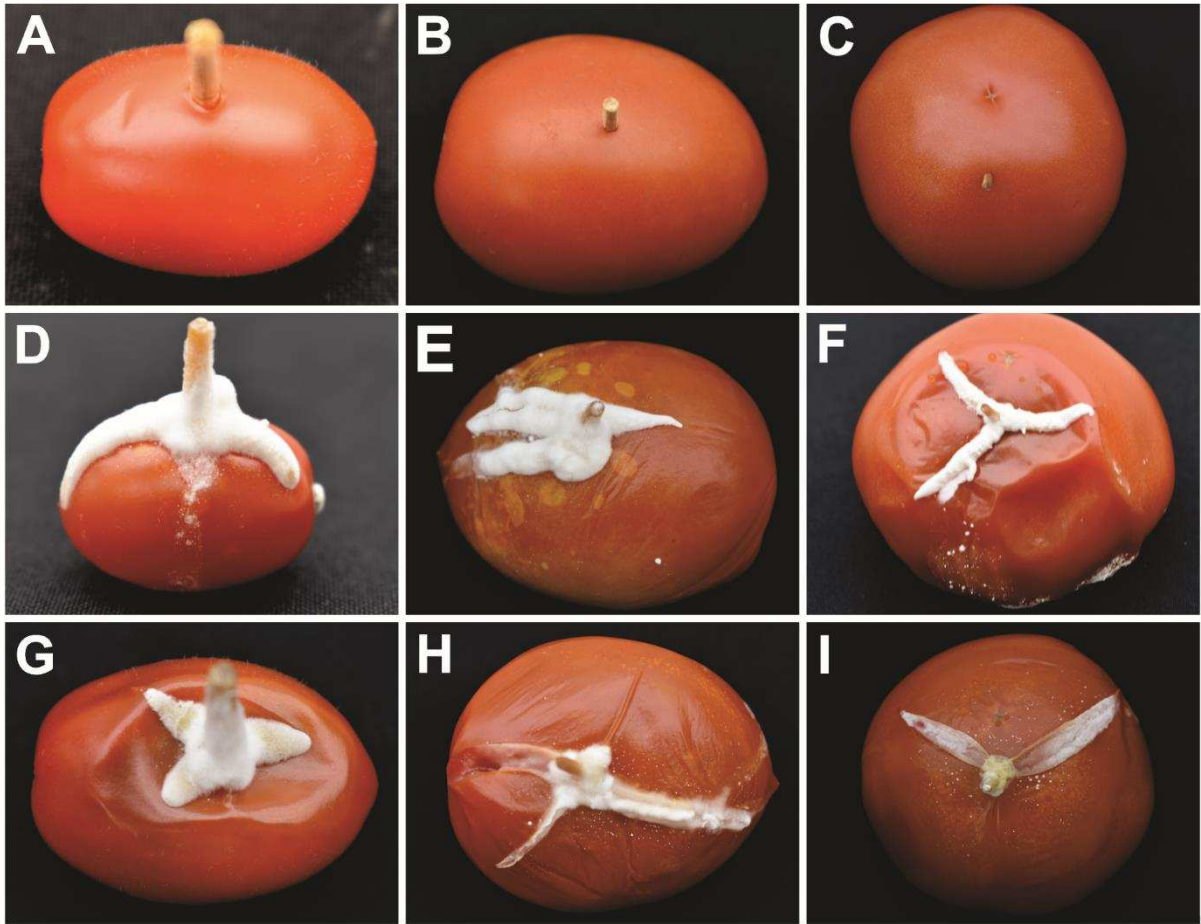
**FIGURE 9.** Pathogenicity test of *Geotrichum* sp. 2 (COAD 2973). **b.** Sour rot on Sweet Grape tomato; **d.** Italian tomato; **f.** Long-shelf Life tomato; **h.** Red mombin fruits.; **a, c, e, g.** Control treatment.



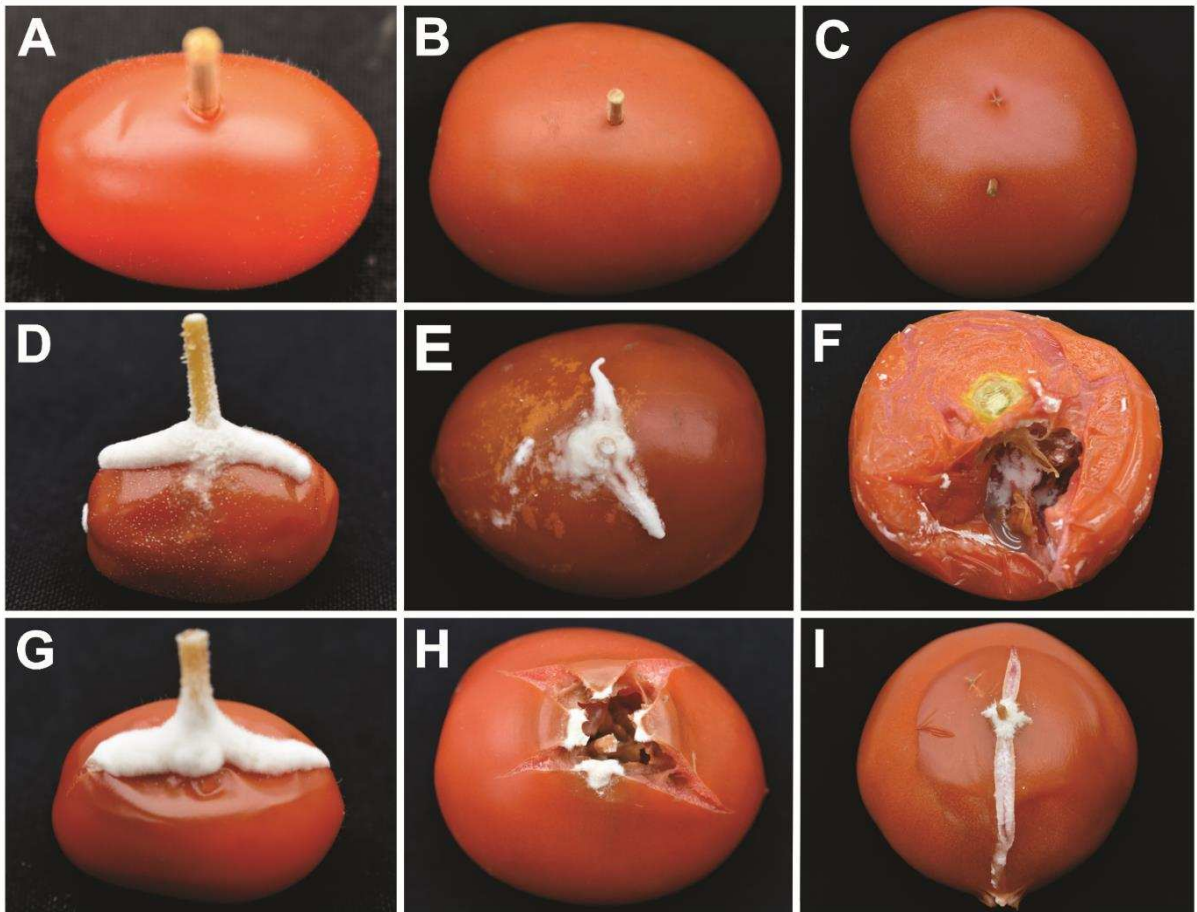
**FIGURE 10.** *Geotrichum* sp. 1 (COAD 2974). **a.** Colony on PDA (Petri dishes with 90 × 15 mm); **b.** Colony on YM (Petri dishes with 90 × 15 mm); **c.** Conidia on PDA; **d.** Conidia on YM; Scale bars: d–e = 10 μm.



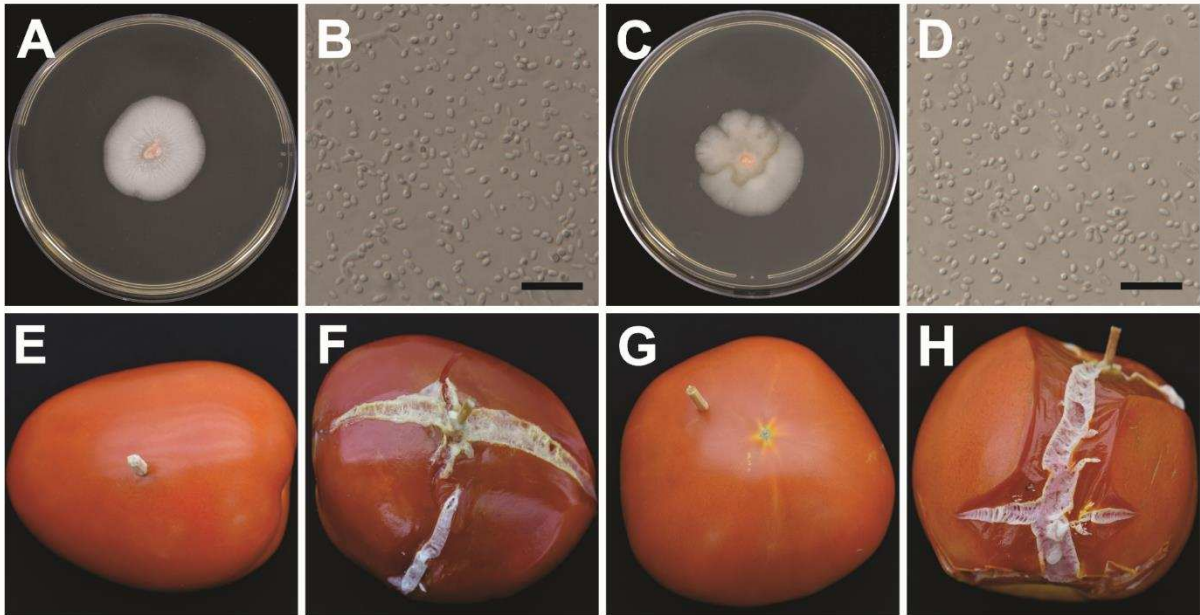
**FIGURE 11.** *Geotrichum* sp. 2 (COAD 2973). **a.** Colony on PDA (Petri dishes with 90 × 15 mm); **b.** Colony on YM (Petri dishes with 90 × 15 mm); **c.** Conidia and hyphae on PDA; **d.** Conidia and hyphae on YM; Scale bars: b–d = 10 μm.



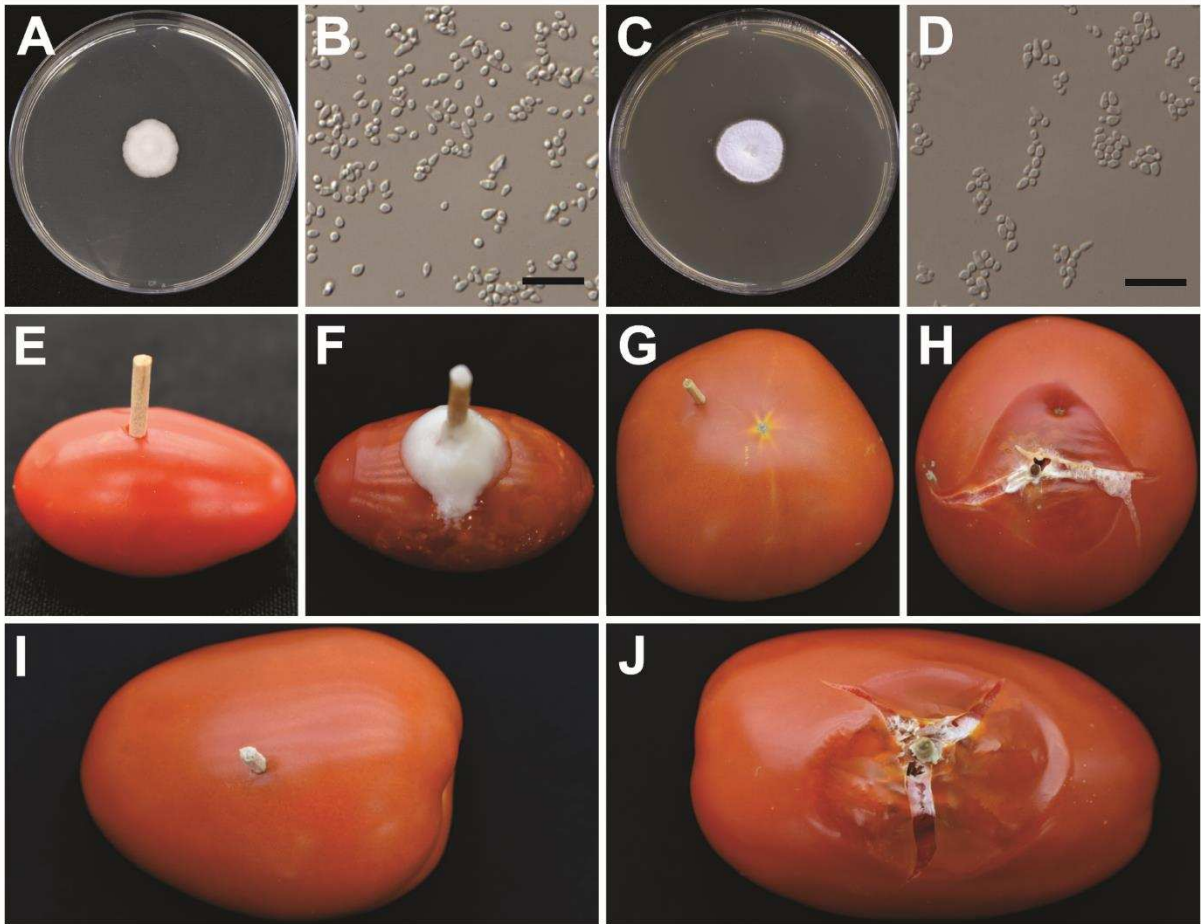
**FIGURE 12:** Pathogenicity test at 25 °C. **a, b, c.** Control treatment. **d, e, f.** Sour rot by *Geotrichum candidum* COAD 2969 on Sweet Grape tomato, Italian tomato, Long-shelf Life tomato respectively; **g, h, i.** Sour rot by *Geotrichum phurueaensis* COAD 2970 on Sweet Grape tomato, Italian tomato, Long-shelf Life tomato, respectively.



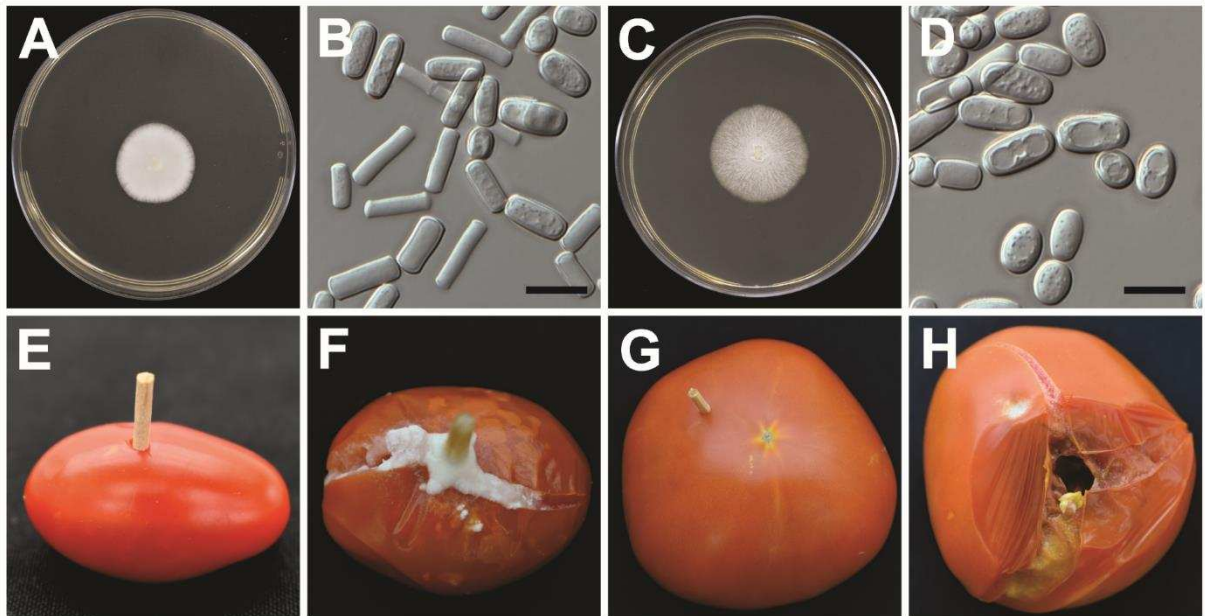
**FIGURE 13:** Pathogenicity test at 25 °C. **a, b, c.** Control treatment. **d, e, f.** Sour rot by *Geotrichum citri-aurantii* COAD 2971 on Sweet Grape tomato, Italian tomato, Long-shelf Life tomato respectively; **g, h, i.** Sour rot by *Geotrichum vulgare* COAD 2975 on Sweet Grape tomato, Italian tomato, Long-shelf Life tomato respectively.



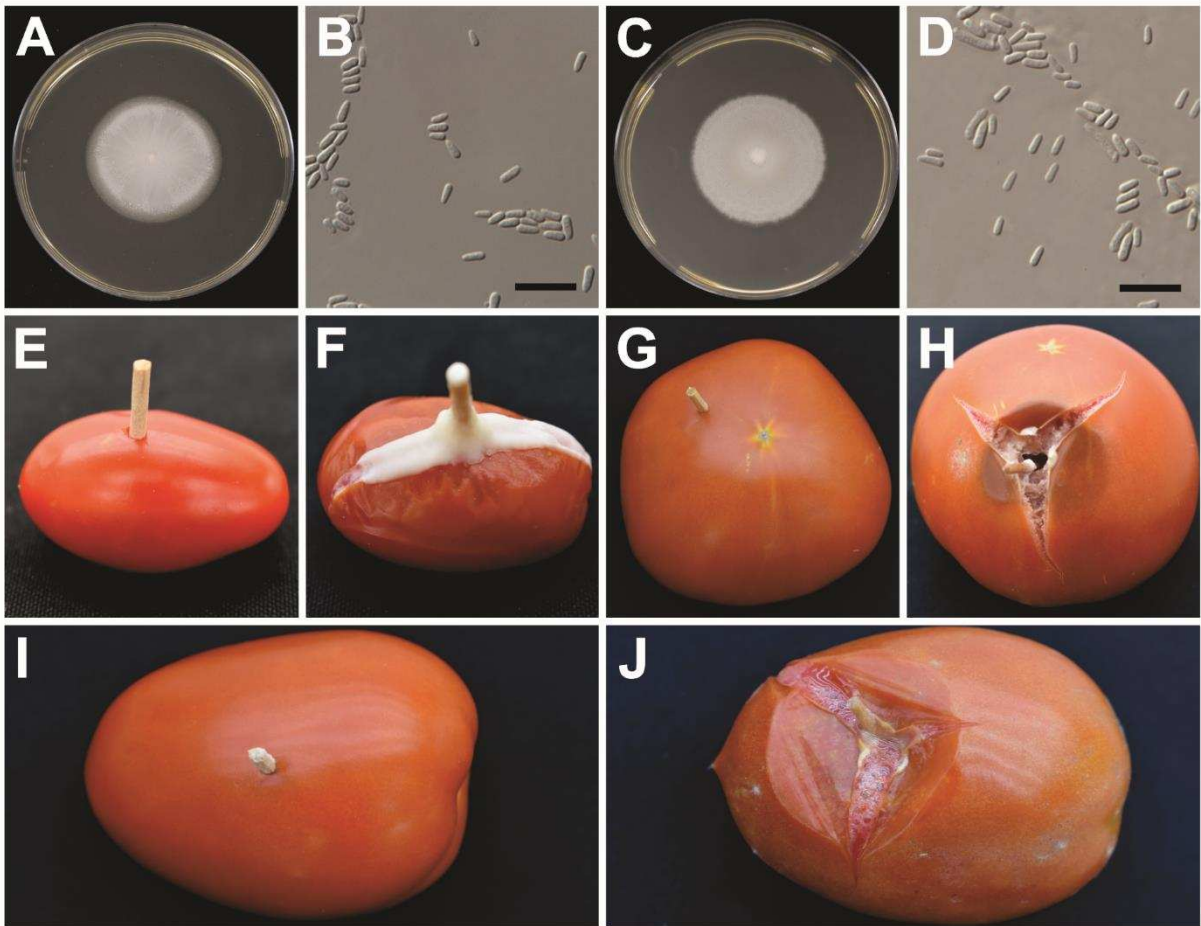
**FIGURE 14.** Pathogenicity test of *Hyphopichia khmerensis* CDA 2446. **a.** Colony on PDA (Petri dishes with 90 × 15 mm); **b.** Conidia on PDA; **c.** Colony on YM (Petri dishes with 90 × 15 mm); **d.** Conidia on YM; **f.** Sour rot on Sweet Grape tomato; **h.** Long-shelf Life tomato; **j.** Italian tomato; **e, g.** Control treatment. Scale bars: b-d = 10 μm.



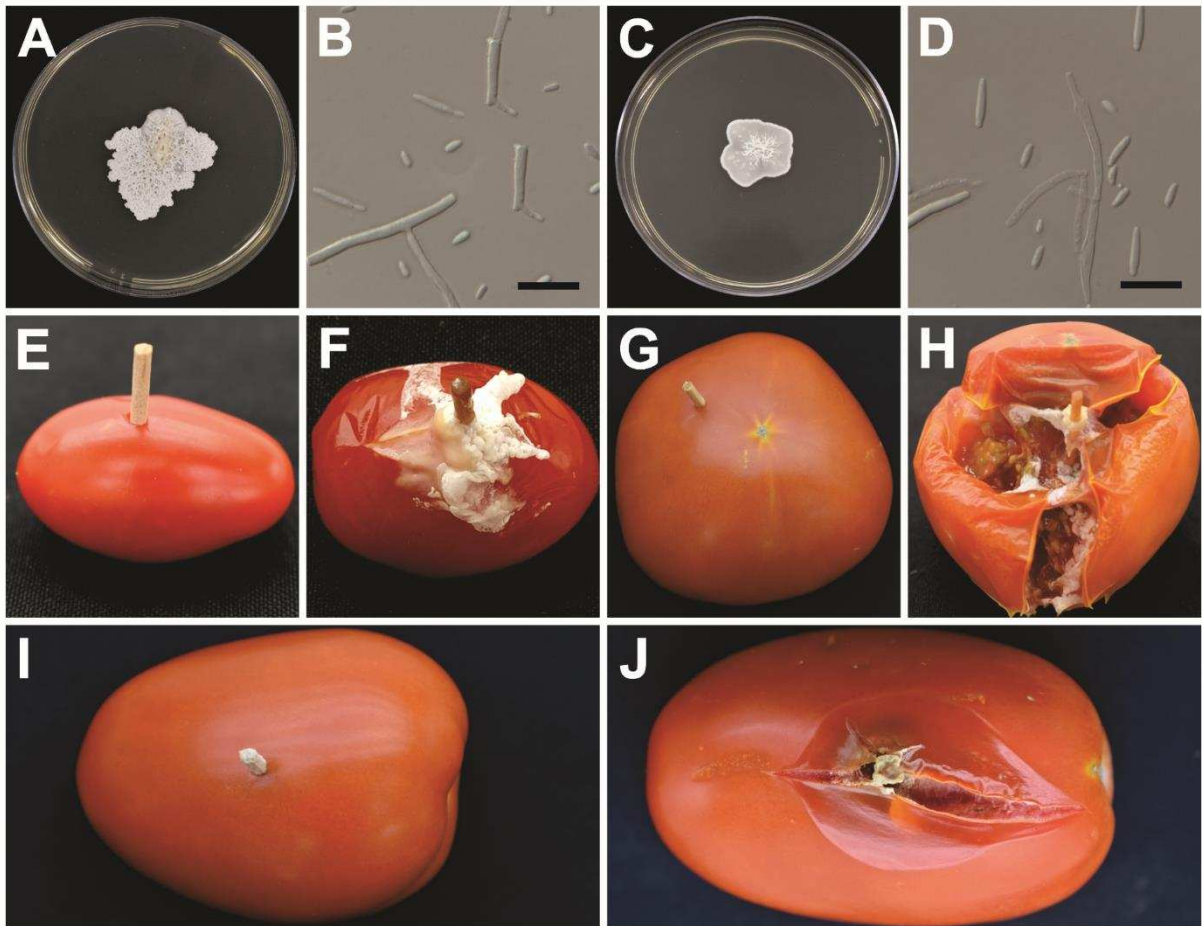
**FIGURE 15.** Pathogenicity test of *Hyphopichia burtonii* CDA 1969. **a.** Colony on PDA (Petri dishes with 90 × 15 mm); **b.** Conidia on PDA; **c.** Colony on YM (Petri dishes with 90 × 15 mm); **d.** Conidia on YM; **f.** Sour rot on Sweet Grape tomato; **h.** Long-shelf Life tomato; **j.** Italian tomato; **e, g, i.** Control treatment. Scale bars: b–d = 10 μm.



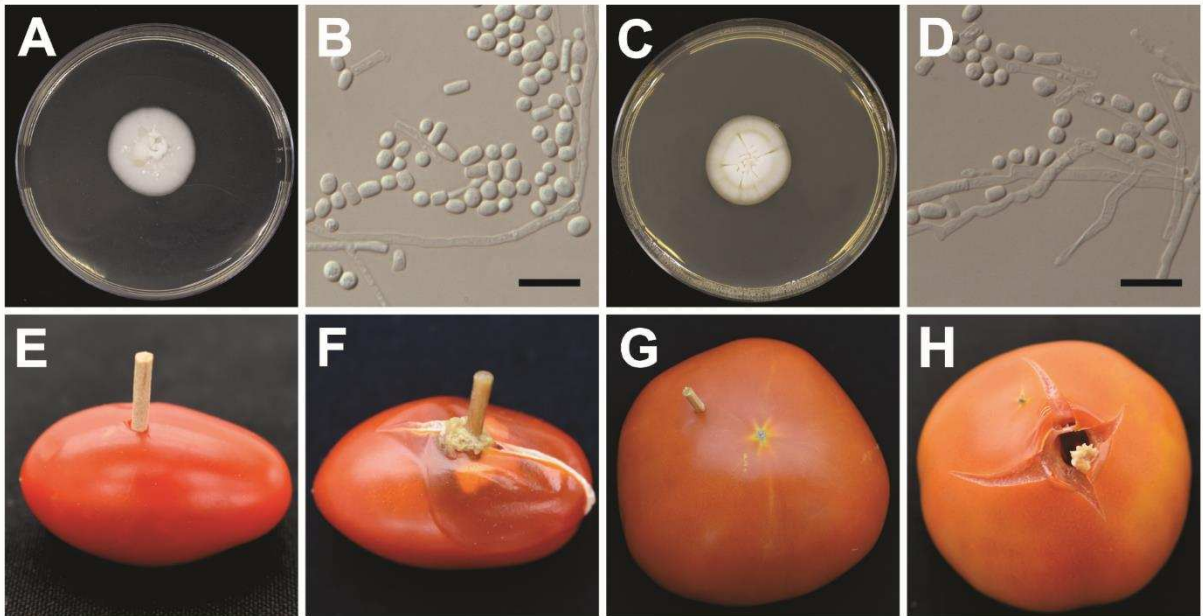
**FIGURE 16.** Pathogenicity test of *Magnusiomyces tetrasperma* CDA 2097. **a.** Colony on PDA (Petri dishes with 90 × 15 mm); **b.** Conidia on PDA; **c.** Colony on YM (Petri dishes with 90 × 15 mm); **d.** Conidia on YM; **f.** Sour rot on Sweet Grape tomato; **h.** Long-shelf Life tomato; **e,** **g.** Control treatment. Scale bars: b–d = 10 μm.



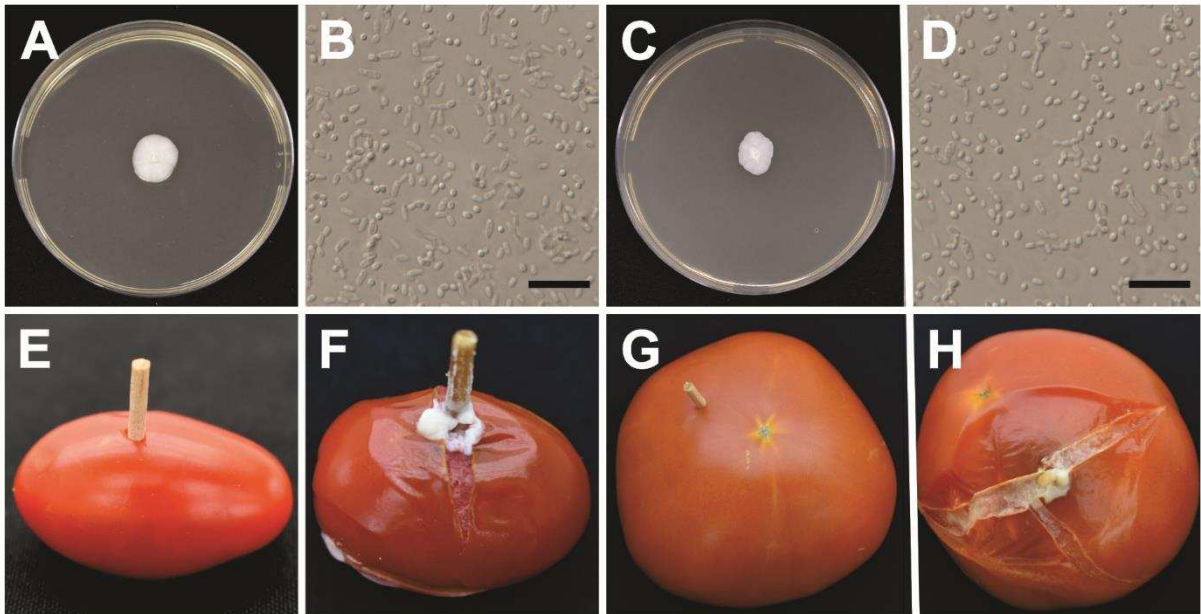
**FIGURE 17.** Pathogenicity test of *Saccharomycopsis vini* CDA 2041. **a.** Colony on PDA (Petri dishes with 90 × 15 mm); **b.** Conidia on PDA; **c.** Colony on YM (Petri dishes with 90 × 15 mm); **d.** Conidia on YM; **f.** Sour rot on Sweet Grape tomato; **h.** Long-shelf Life tomato; **j.** Italian tomato; **e, g, i.** Control treatment. Scale bars: b–d = 10 μm.



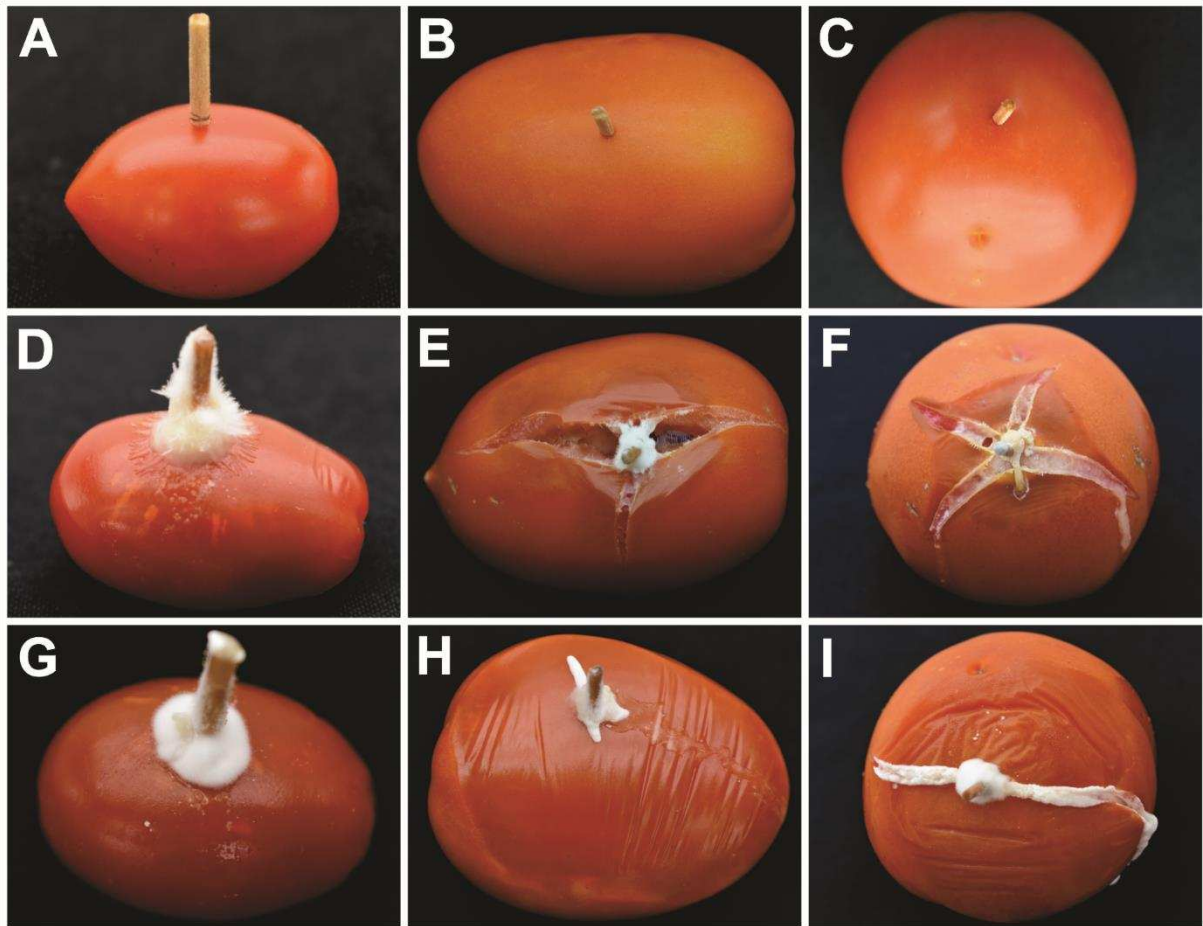
**FIGURE 18.** Pathogenicity test of *Saccharomycopsis crataegensis* CDA 2455. **a.** Colony on PDA (Petri dishes with 90 × 15 mm); **b.** Conidia on PDA; **c.** Colony on YM (Petri dishes with 90 × 15 mm); **d.** Conidia on YM; **f.** Sour rot on Sweet Grape tomato; **h.** Long-shelf Life tomato; **j.** Italian tomato; **e, g, i.** Control treatment. Scale bars: b-d = 10 μm.



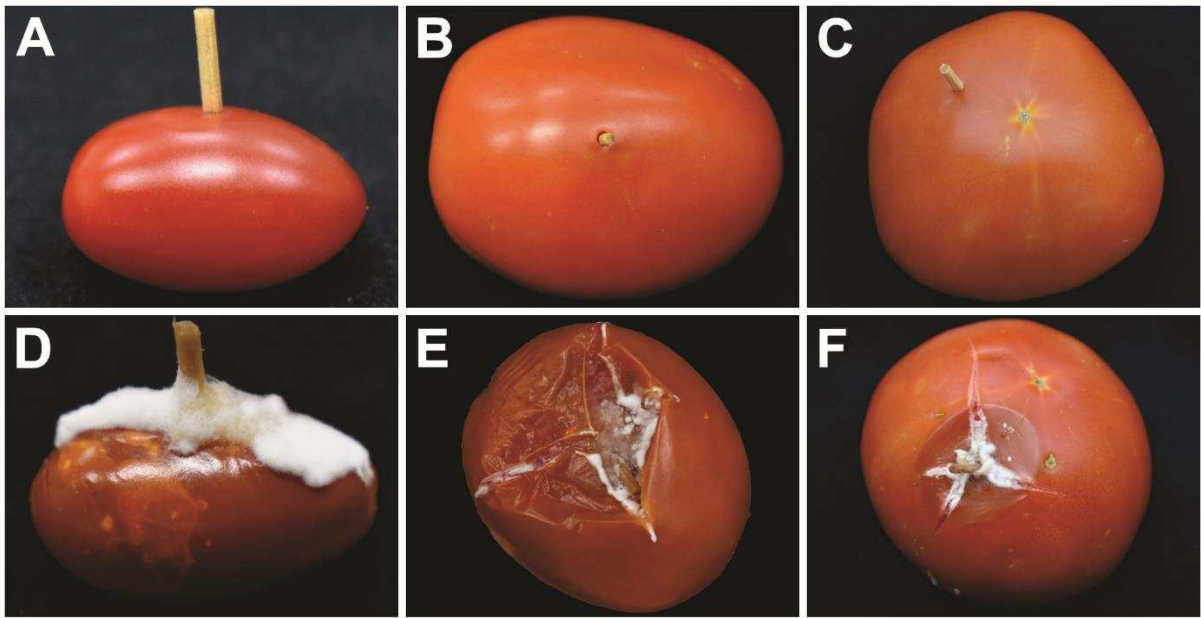
**FIGURE 19.** Pathogenicity test of *Trichosporon coremiiforme* CDA 2459. **a.** Colony on PDA (Petri dishes with 90 × 15 mm); **b.** Conidia on PDA; **c.** Colony on YM (Petri dishes with 90 × 15 mm); **d.** Conidia on YM; **f.** Sour rot on Sweet Grape tomato; **h.** Long-shelf Life tomato; **e, g.** Control treatment. Scale bars: b-d = 10 μm.



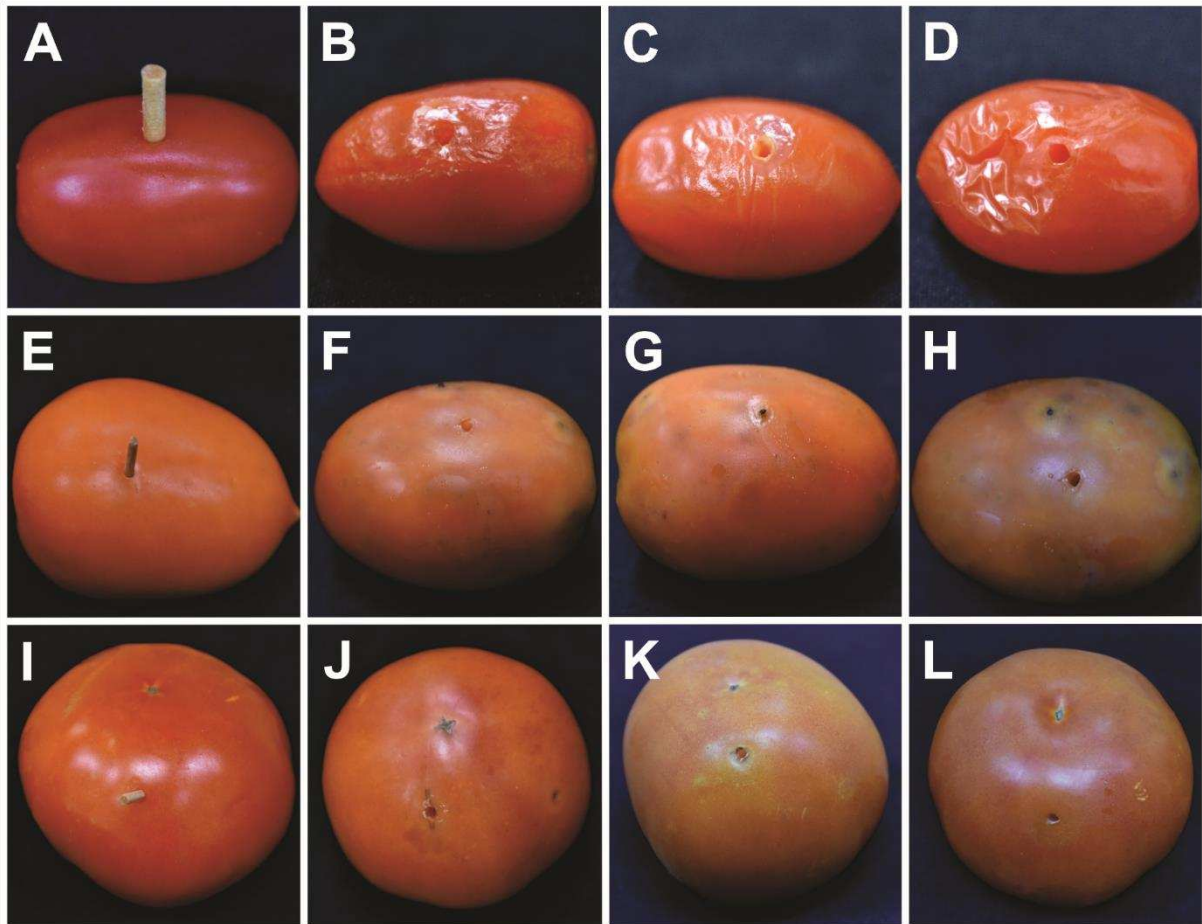
**FIGURE 20.** Pathogenicity test of *Zygoascus meyeriae* CDA 2463. **a.** Colony on PDA (Petri dishes with 90 × 15 mm); **b.** Conidia on PDA; **c.** Colony on YM (Petri dishes with 90 × 15 mm); **d.** Conidia on YM; **f.** Sour rot on Sweet Grape tomato; **h.** Long-shelf Life tomato; **e, g.** Control treatment. Scale bars: b-d = 10 μm.



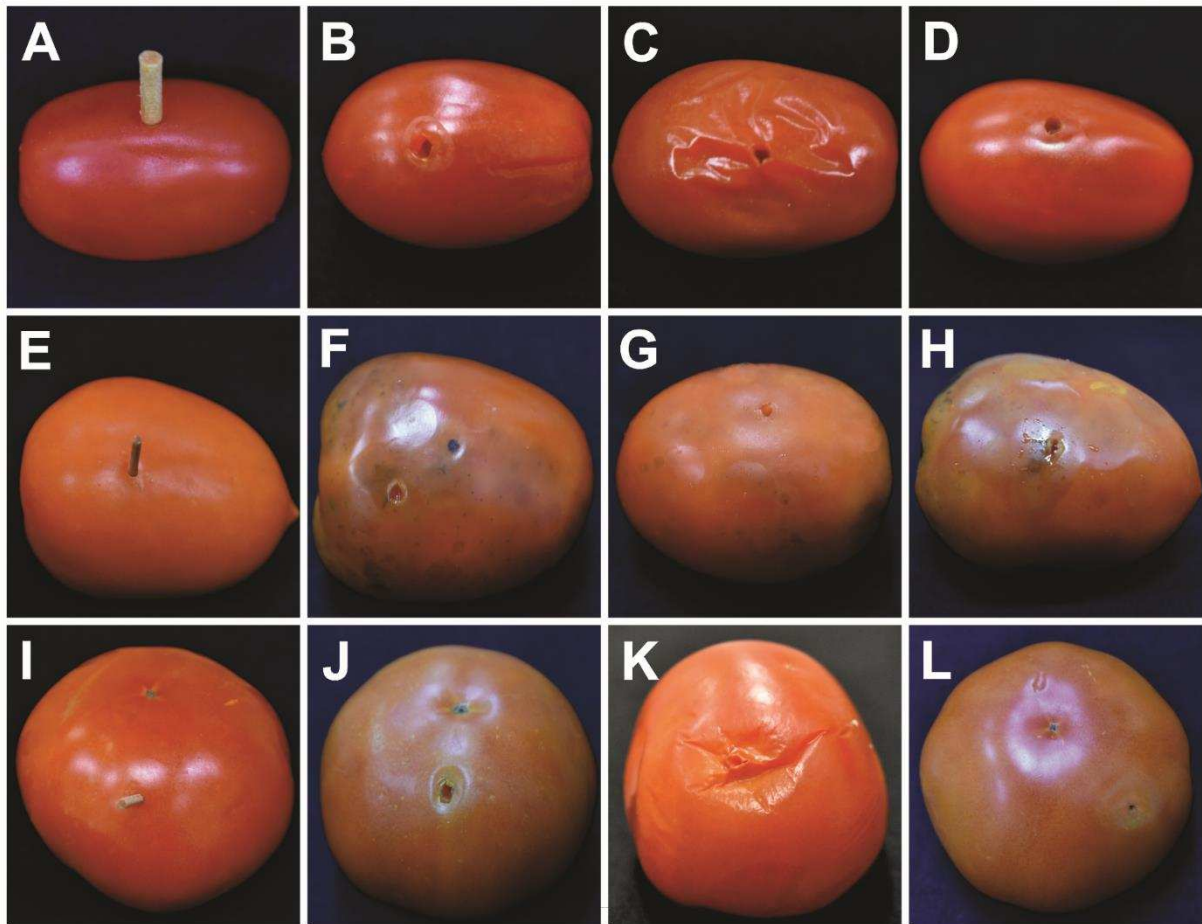
**FIGURE 21.** Pathogenicity test at 19 °C. **a, b, c.** Control treatment. **d, e, f.** Sour rot by *Geotrichum sp. 2* COAD 2972 on Sweet Grape tomato, Italian tomato, Long-shelf Life tomato respectively; **g, h, i.** Sour rot by *Geotrichum candidum* COAD 2969 on Sweet Grape tomato, Italian tomato, Long-shelf Life tomato respectively.



**FIGURE 22.** Pathogenicity test at 22 °C. **a, b, c.** Control treatment. **d, e, f.** Sour rot by *Geotrichum vulgare* COAD 2975 on Sweet Grape tomato, Italian tomato, Long-shelf Life tomato respectively.



**FIGURE 23.** Pathogenicity test at 8 °C. **a, e, i.** Control treatment. **b, f, j.** Sour rot by *Magnusiomyces tetrasperma* COAD 2097. **c, g, k.** *Geotrichum candidum* COAD 2969. **d, h, l.** *Geotrichum phurueaensis* COAD 2970 on Sweet Grape tomato, Italian tomato, Long-shelf Life tomato.



**FIGURE 24.** Pathogenicity test at 8 °C. **a, e, i.** Control treatment. **b, f, j.** Sour rot by *Geotrichum* sp. 2 COAD 2972; **c, g, k.** *Geotrichum* sp. 1 COAD 2974; **d, h, l.** *Geotrichum vulgare* COAD 2975 on Sweet Grape tomato, Italian tomato, Long-shelf Life tomato.