VOLUME 9 NOMOR 1 JUNI 2022 **ISSN 2548 – 611X**

JURNAL
BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejurnal.bppt.go.id/index.php/JBBI

INHIBITORY ACTIVITY OF *Trichoderma harzianum* AGAINST PUTATIVELY PATHOGENIC FUNGUS ON RODENT TUBER (*Typhonium flagelliforme*) PLANT

Aktifitas Penghambatan *Trichoderma harzianum* terhadap Kapang Terduga Patogen Pada Tanaman Keladi Tikus (*Typhonium flagelliforme*)

Rantika Silfarohana, Aji Wibowo, Nia Asiani, Zhafira Amila Haqqa, Mahmud Sugianto, Catur Sriherwanto*
National Research and Innovation Agency (BRIN), Science and Technology Park,
South Tangerang, Banten, Indonesia
*Email: catur.sriherwanto@brin.go.id

ABSTRACT
Trichoderma *spp. are globally considered as the most dominant biofungicide in the market. Reports on* Trichoderma *spp. efficacy against pathogenic fungi in commercial crops have been numerous, but much less in medicinal plants. This study aimed at testing the potential biofungicidal activity of* Trichoderma harzianum *in inhibiting the growth of a putatively pathogenic fungus isolated from rodent tuber (*Typhonium flagelliforme*) plant. The methods consisted of isolation of fungi from the plant's surface, soil, and polybags. The isolates were then screened for their putative pathogenicity against rodent tuber before being subjected to 16S rRNA molecular identification and in vitro antagonist test using* T. harzianum*. Result showed that only isolate K4 showed pathogenicity on* T. flagelliforme, *and was molecularly identified as* Lasiodiplodia theobromae*, known globally as fungal pathogen attacking various plants.* L. theobromae *was inhibited by* T. harzianum *with inhibition index of 23.0 ± 4.3%, which was about twice higher than that of the positive control nystatin 100.000 IU mL (11.1 ± 0.6%). In conclusion,* T. harzianum *inhibited the growth of* L. theobromae in vitro*, hence indicating its biofungicidal potential.*

Keywords: *biofungicide,* Lasiodiplodia theobrom*,* Trichoderma harzianum*,* Typhonium flagelliforme***,*** *pathogen*

ABSTRAK
Trichoderma spp. merupakan biofungisida paling dominan di pasar global. Kemampuan pengendalian menggunakan *Trichoderma* spp. terhadap kapang patogen pada tanaman komoditas pangan sudah banyak dilaporkan, namun belum banyak untuk tanaman obat. Penelitian ini bertujuan menguji potensi aktivitas biofungisida *Trichoderma harzianum* dalam menghambat pertumbuhan kapang terduga patogen yang diisolasi dari tanaman keladi tikus (*Typhonium flagelliforme*). Metode penelitian meliputi isolasi kapang dari permukaan tanaman, tanah, dan polibag. Penapisan dilakukan untuk mendapatkan isolat yang berpotensi patogen terhadap keladi tikus, untuk kemudian diidentifikasi secara molekuler menggunakan 16S rRNA dan diuji antagonis in vitro menggunakan *T. harzianum*. Hasil penelitian menunjukkan bahwa hanya isolat K4 yang bersifat patogen pada *T. flagelliforme* dan secara molekuler diidentifikasi sebagai *Lasiodiplodia theobromae*, yang dikenal sebagai jamur patogen yang menyerang berbagai tanaman. Pertumbuhan *L. theobromae* dihambat oleh *T. harzianum* dengan indeks penghambatan 23,0 ± 4,3%, atau dua kali lebih tinggi dari kontrol positif nistatin 100.000 IU mL (11,1 ± 0,6%). Sebagai kesimpulan, *T. harzianum* menghambat pertumbuhan *L. theobromae* secara *in vitro*, yang menunjukkan potensi biofungisidanya.

Kata Kunci: biofungisida*, Lasiodiplodia theobrom, Trichoderma harzianum, Typhonium flagelliforme***,** patogen

Received: *13 January 2022* ***Accepted:*** *15 February 2022* ***Published:*** *01 June 2022*

INTRODUCTION

At present, biofungicides containing *Trichoderma* spp. are considered as the commercially most dominant biofungicide products in the world's biopesticide market (Meher et al. 2020). Their efficacy has been demonstrated recently against plant pathogenic fungi including *Magnaporthiopsis maydis* in maize (Degani and Dor 2021), *Erysiphe alphitoides* in common oak (Oszako et al. 2021), *Fusarium incarnatum* in muskmelon (Intana et al. 2021), *Alternaria solani* in potato (Kumar et al. 2021), *Plasmopara viticola* in grapevine (Kamble et al. 2021), *Fusarium solani* in peanut (Erazo et al. 2021), and *Colletotrichum gloeosporioides* in chili pepper (Ruangwong et al. 2021). In addition, current researches on this soil-borne fungal genus has continually been undertaken on aspects related to its use as biocontrol such as mitogenomes (Kwak 2021), combined application with a synthetic fungicide (Zhang et al. 2021), growth promoting effect (Yu et al. 2021), production in solid state fermentation (Liu et al. 2021; Mousumi Das et al. 2021; Sala et al. 2021), and fungal selection against multiple pathosystems (Manganiello et al. 2021).

While numerous researches have been devoted on the use of *Trichoderma* spp. as biofungicide agents against fungal pathogens in economically important crops, fewer reports have been published on their use in medicinal plants (Singh and Pandey 2020). For instance, *Trichoderma viride* was shown to have >60% antagonistic activity *in vitro* against *Alternaria alternata* and *Sclerotium rolfsii*, which are the fungal pathogens of Indian gingseng (*Withania somnifera*) (Kushwaha et al. 2019). The study also reported that, inoculation of both *T. viride* and the native endophytic fungi of *W. somnifera* improved the plant growth and the accumulation of the bioactive compounds withanolides. In another medicinal plant, turmeric, systemic resistance against the rhizome rot causing-fungus *Pythium aphanidermatum* was induced by*Trichoderma asperellum* (Vinayarani et al. 2019). In the study, *T. asperellum* was amongst 5 of 30 fungi isolated from the turmeric rhizosphere which exhibited more than 70% inhibition against *P. aphanidermatum* as well as multiple plant growth promoting activities *in vitro*. Similar results were obtained by Huang et al. (2021) who used *Trichoderma brevicompactum* to control the root rot disease caused by *Fusarium oxysporum* in the chinese medicinal plant *Atractylodes macrocephala*.

Rodent tuber (*Typhonium flagelliforme*) is a medicinal plant commonly found in Indonesia and known for its anticancer (Khalivulla et al. 2019) and antioxidant (Septaningsih et al. 2021) activities. There have been numerous studies on the *in vitro* propagation of rodent tuber plant, but not on its large-scale cultivation, which is very rare. In one of such cultivation study, identifying the plant's pathogen was deemed important since pathogenic attack could destroy all the leaves of a rodent tuber clump with more than 20 shoots in a short time (Juhaeti 2002). Since medicinal plants are not different from other plants in that they have specific fungal pathogens (Abtahi and Nourani 2017), the genus *Typhonium* could have them too. Thus, this study aimed at testing the potential biofungicide *Trichoderma harzianum* for its inhibitory activity against the growth of a putatively pathogenic fungus isolated from rodent tuber (*Typhonium flagelliforme*) plant.

MATERIALS AND METHODS

Location and time

This study was conducted in January 2021–January 2022 at the Biotechnology Laboratory, the National Research and Innovation Agency (BRIN), Science and Technology Park, South Tangerang, Banten, Indonesia. Molecular identification was carried out by Genetika Science Indonesia Ltd. (Tangerang City, Banten).

Isolation of putatively pathogenic fungi

Since no report has been published on pathogenic fungi known to attack *T. flagelliforme* cultivated outdoor in the field, this study started with isolating the putative pathogenic fungi of the medicinal plant *T. flagelliforme* (Figure 1) available in the medical plant collection of the Biotechnology Laboratory, the National Research and Innovation Agency (BRIN), Science and Technology Park, South

Tangerang, Banten, Indonesia. Fungal isolation was carried out from the surface of the stems, soil, and the inner surface of the polybags containing the plants, specifically on spots showing mycelial formation and/or plant lesions, as well as from aphids living on the plants. The isolates were taken directly using a sterile ose needle and then transferred to glass Petri dishes (9 cm diameter) containing selective media for fungal growth, namely Sabouraud Dextrose Agar (SDA, Oxoid, UK), Malt Extract Agar (MEA, Oxoid, UK), and Potato Dextrose Agar (PDA, Oxoid, UK). Incubation was carried out for 5–7 days at room temperature. The fungal isolates cultured on the Petri dishes were observed, and marked based on different morphologies. When it was identified as not purely single culture, the isolates were further purified by subculturing on PDA medium at room temperature for 5–7 days or until fungal colonies were observed. The pure isolates were examined macroscopically and microscopically at 10× and 40× magnification for morphological identification. If, following the observation, the isolates were deemed still not pure, the isolates were purified further using the same procedure. Macroscopic characterization was carried out on the colony morphology and colours, whereas microscopic characterization on the shapes of hyphae and spores or conidia. Subculture of pure isolates was carried out by transferring 1 × 1 cm agar cut containing the mycelia onto a new PDA medium, incubated at room temperature until the fungal colony formed.

Figure 1. Medicinal plant rodent tuber (*T. flagelliforme*)

Pathogenicity test

Pathogenicity test was carried out in order to know whether the individual fungal isolates, which were deliberately inoculated onto the intact (undamaged) leaf and stem surfaces of the rodent tuber plant, were able to grow on the living rodent tuber plant, causing infection, disease, and lesion. The rodent tuber plants to be infected (or inoculated) with the fungal isolates were prepared 2 weeks prior to the pathogenicity test. Medium containing a mixture of soil, husk charcoal, and sand in 2:1:1 ratio was sieved, steam sterilised for 2 hours, and transferred into 15 × 15 cm polybags. These polybags were used to cultivate rodent tuber plants (2–4 months old) which were taken from previous collection. Before planted into the new polybags, the plants were surface sterilized by washing with tap water, spraying with 70% ethanol, and finally rinsing using sterilized reverse osmosis (RO) water.

For the inoculation of the putatively pathogenic fungal isolates onto the tuber rodent plants, the pure fungal isolates were regenerated by subculturing onto PDA Petri dishes, incubated at room temperature for 5 days. This PDA subcultures were used to inoculate 50 mL Erlenmeyer flasks containing 10 mL potato dextrose broth (PDB, Difco, USA), followed by 10-day incubation at room temperature, shaking at 150 rpm. The mycelial mass yielded were then crushed using spatula to reduce its size so as to produce mycelium suspension which ease inoculation onto the host plants. All of the procedures were done aseptically.

To test if the fungal isolates could cause disease to the plant, the fungal mycelium suspension was applied to the surface of rodent tuber leaves and stems. Using a cotton bud, the mycelium suspension was smeared onto the intact (undamaged) leaf and stem surfaces which

were previously sterilized using 70% ethanol. For each fungal isolate, each treatment was repeated 3 times. After inoculation, the plants were placed at 25-cm distance apart from each other in an orderly arrangement based on the completely randomized design. Observation of the signs of disease (wilts, yellowing, brown streaks, leaf spots, and chlorosis) on the inoculated plants was carried out for 7 days.

Re-isolation of putatively pathogenic fungi

The fungal-inoculated rodent tuber plants were identified for the signs of infection. The leaves and/or stems showing disease symptoms were cut off, cleaned and surface sterilized by firstly removing the dirt with running tap water for about 10 seconds. These samples were then cut into 1 × 1 cm size, soaked in 70% ethanol for 30 seconds, and subsequently drained to dry. Next, the samples were rinsed using sterile RO water, soaked in 5.3% sodium hypochlorite for 30 seconds, rinsed again with sterile RO water, drained and dried using sterile white tissue paper. These surfaced sterilised samples were grown on PDA agar in Petri dishes (9 cm diameter). The growing fungi were isolated and identified macroscopically and microscopically, and compared their morphological similarity with those fungi initially used to infect the plant.

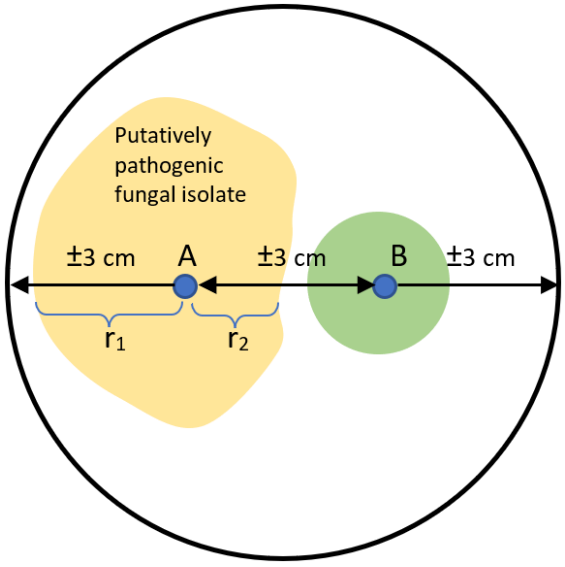

Figure 2. Antagonist test on PDA Petri dishes: A. Inoculation point of putatively pathogenic fungal isolate; B. Inoculation point of potential biofungicide fungus (*T. harzianum*) or placement point of either sterile RO water (negative control) or nystatin (positive control).

16S rRNA Molecular Identification

One fungal isolate, which was confirmed to have pathogenic trait on *T. flagelliforme*, was sent to Genetika Science Indonesia Ltd. (Tangerang City, Banten) for molecular identification using 16S rRNA as marker. The procedure started with genomic DNA extraction using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005, USA), followed by PCR amplification using (2×) MyTaq HS Red Mix (Bioline, BIO-25048, UK) and ITS 1-4 primer. Finally, bi-directional sequencing was then carried out. The resulting DNA sequence was analysed using BLAST against the NCBI 16S rDNA database (https://blast.ncbi.nlm.ih.gov/Blast.cgi).

Antagonist test

Double-well inhibition zone assay was used to test the efficacy of biofungicide fungal candidate *Trichoderma harzianum* against the putatively pathogenic fungal isolate. To do this, PDA on disposable Petri dishes (8.7 cm diameter) were prepared in which two identical wells were made on the agar surface, along the diameter line. Both wells were approximately of ±3 cm apart and each well was of ±3-cm distance from the Petri dish edge (Figure 2). One well was for inoculation of the putatively pathogenic fungal isolate and the other was either for sterile RO water (negative control), nystatin 100.000 IU mL (positive control), or *T. harzianum*. A volume of 0.1 mL was used for transferring the sterile RO water or nystatin aqueous solution into the two wells. *T. harzianum* mycelium, initially subcultured on PDA, was transferred into the well by single-point inoculation. Each treatment was replicated twice and was incubated at room temperature (25–27 °C) for 7 days, during which the fungal growth was observed and the inhibition index was calculated using the following formula:

$$Inhibition\ Index\ (\%) = \frac{(r_1 - r_2)}{r_1} \times 100\%$$

Where: r_1 and r_2 (in mm) represent the colony radii of the putatively pathogenic fungal isolate toward the Petri edge and toward the inhibiting agent, respectively.

Table 1. Putatively pathogenic fungi isolated from rodent tuber plant (*T. flagelliforme*)

No	Fungal Isolate Code	Result of Identification	Pathogenicity Test
1	B1	Unidentified	tested
2	B2	Unidentified	tested
3	B3	*Gliomastix murorum*	untested
4	B4	*Rhizopus* sp.	untested
5	B5	*Rhizopus* sp.	untested
6	B6	Unidentified	tested
7	B7	*Penicillium* sp.	tested
8	B8	Unidentified	tested
9	D4	*Aspergillus* sp.	untested
10	D5	*Aspergillus* sp.	untested
11	D8	Unidentified	untested
12	D9	Unidentified	tested
13	D10	*Mycogone*	tested
14	T2	*Aspergillus* sp.	untested
15	T3	Unidentified	tested
16	T4	Unidentified	tested
17	K1	*Geotrichum*	tested
18	K2	*Aspergillus* sp.	untested
19	K4	Unidentified	tested
20	K5	*Penicillium citrinum*	untested
21	T.As.k	Unidentified	tested
22	T(K)2MT	Unidentified	tested
23	BT(K)3	Unidentified	tested

RESULTS AND DISCUSSION

Putatively pathogenic fungal isolates

In total, 23 fungal isolates (Table 1) were obtained, some of which were macroscopically and microscopically identified as *Penicillium* (2 isolates), *Aspergillus* sp. (4 isolates), *Gliomastix murorum* (1 isolate), *Geotrichum* (1 isolate), *Mycogone* (1 isolate), and *Rhizopus* sp. (2 isolates), whereas the other 12 isolates were unidentified. These isolates were further subjected to pathogenic test, with the exception of fungal isolates commonly found in soil (such as *Rhizopus* and *Aspergillus*), known as non-pathogenic to plants (*Gliomastixm, Mycogone,* and *Geotrichum*), and those isolates that could not be regenerated.

Pathogenicity test

As many as 14 isolates were used in pathogenicity test against the rodent tuber plants. Based on the results of pathogenicity test (Table 2), the inoculation

Table 2. Putatively fungal isolates inoculated on rodent tuber plant

No	Fungal Isolate Code	Observable Symptoms on	
		Leaves	Stems
1	B1	no	no
2	B2	yes	no
3	B6	no	no
4	B7	no	no
5	B8	no	no
6	D9	yes	no
7	D10	no	no
8	T3	yes	no
9	T4	yes	no
10	K1	no	no
11	K4	yes	no
12	T.As.k	yes	no
13	T(K)2MT	yes	no
14	BT(K)3	yes	yes

of 14 isolates showed only 8 isolates (B2, D9, T3, T4, K4, T.As.k, T(K)2MT, and BT(K)3) which produced symptoms on the leaves of the inoculated rodent tuber plants. In contrast, only those plants inoculated with BT(K)3 displayed symptom on the stems. To confirm whether it was the initially inoculated fungal isolates that caused the disease symptoms, re-isolation was then carried out from the symptomatic plants. It was found that it was only isolate K4 that showed morphological similarity with the fungus re-isolated from the plant previously inoculated with isolate K4. Thus, K4 was designated as the putatively pathogenic fungus for rodent tuber plant grown in green house.

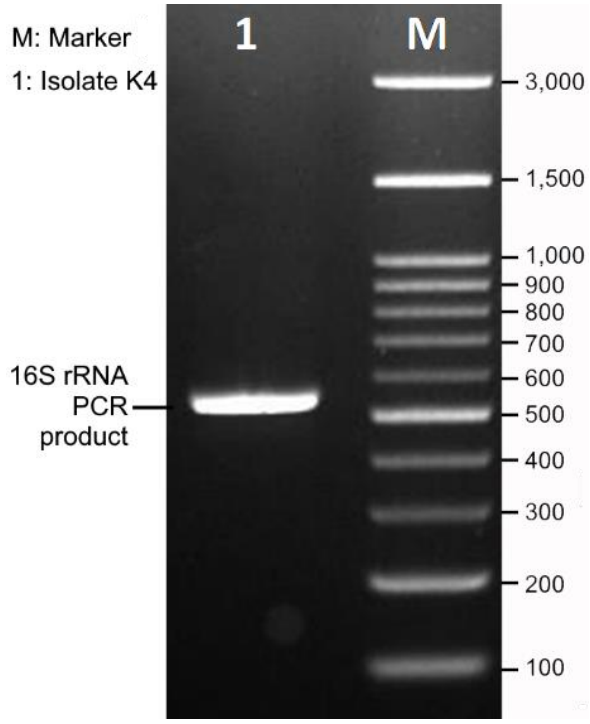

Figure 3. PCR products of 16S rRNA extracted from isolate K4

Molecular identification

The PCR product of 16S rRNA extracted from isolate K4 produced a single bright band on agarose gel, showing the size of between 500-600 bp (Figure 3). Sequencing and its subsequent alignment analysis of the sequence assembly result (Figure 4) using BLAST against the NCBI 16S rDNA database showed that isolate K4 was 99–100% identical to the fungus *Lasiodiplodia theobromae* (Figure 5).

Isolate K4 was molecularly identified as *Lasiodiplodia theobromae*, which is known to be pathogenic to crops and woody plants around the world, including cacao (Ali et al. 2019), tea (Jiang et al. 2020), mahogany (Webber et al. 2021), citrus (Zheng et al. 2020), grapevine (Zhang et al. 2019), mango (Kamil et al. 2018), longan fruit (Chen et al. 2021), and coconut palm (Santos et al. 2020). Although *L. theobromae* is rarely reported to cause disease on medicinal plants, there is at least one study in Brazil which mentioned the pathogenic fungus causing ginger rhizome rot (Moreira et al. 2013). This study is the first report on *L. theobromae* likely to be pathogenic to the medicinal plant *T. flagelliforme*. In another study, *L. theobromae* was reported to be the medicinal plant *Morinda citrifolia*'s endophytic fungus, which produces taxol, a compound with anticancer activity (Pandi et al. 2011).

Antagonistic test

In vitro test on PDA dishes showed that *T. harzianum* had the ability to inhibit the growth of the fungal isolate K4 (*L. theobromae)*, which overgrew the entire surface of PDA plate in the absence of inhibitory agent such as nystatin (Figure 6). The inhibition index of *T. harzianum* against *L. theobromae* was 23.0 ± 4.3%, which was

```
1    TGATCCTTCC GTAGGTGAAC CTGCGGAAGG ATCATTACCG AGTTTTCGAG CTCCGGCTCG
61   ACTCTCCCAC CCTTTGTGAA CGTACCTCTG TTGCTTTGGC GGCTCCGGCC GCCAAAGGAC
121  CTTCAAACTC CAGTCAGTAA ACGCAGACGT CTGATAAACA AGTTAATAAA CTAAAACTTT
181  CAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC AGCGAAATGC GATAAGTAAT
241  GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CCCTTGGTAT
301  TCCGGGGGGC ATGCCTGTTC GAGCGTCATT ACAACCCTCA AGCTCTGCTT GGAATTGGGC
361  ACCGTCCTCA CTGCGGACGC GCCTCAAAGA CCTCGGCGGT GGCTGTTCAG CCCTCAAGCG
421  TAGTAGAATA CACCTCGCTT TGGAGCGGTT GGCGTCGCCC GCCGGACGAA CCTTCTGAAC
481  TTTTCTCAAG GTTGACCTCG GATCAGGTAG GGATACCCGC TGAACTTAAG CATATCAATA
541  AGCGGAG
```

Figure 4. Sequence assembly result of the PCR product (547 bp) of 16S rRNA extracted from isolate K4

about twice as much as nystatin 100.000 IU mL (11.1 ± 0.6%).

This study reported the effectivity of *T. harzianum* in inhibiting the growth of pathogenic fungus *L. theobromae* isolated from the medicinal plant *T. flagelliforme*. Similar result was obtained by Wanjiku et al. (2021), where *T. harzianum* were found to be the most effective controlling agent against *L. theobromae* both *in vitro* and on postharvest avocado fruit. *Trichoderma* spp. were also found capable of inhibiting the growth of *L. theobromae* in relation to its pathogenicity on teak (Borges et al. 2018) and grapevine (Rusin et al. 2021). Other *in vitro* growth inhibition of *L. theobromae* by *Trichoderma* was also observed (Bhadra et al. 2015, Dissanayak et al. 2021). Thus, genus *Trichoderma* offers opportunities for further studies on its application to control *L. theobromae* which may attack rodent tuber plants in the field.

CONCLUSION

Trichoderma harzianum demonstrated growth inhibition activity against the putatively pathogenic fungus isolated from *T. flagelliforme* at 23.0 ± 4.3% inhibition level, which was about twice stronger than those by the positive control nystatin 100.000 IU mL (11.1 ± 0.6%). This isolate was molecularly identified as *Lasiodiplodia theobromae,* which is known worldwide to attack various species of plants inhabiting tropical and temperate areas.

Sequences producing significant alignments Download New Select columns Show 10

select all *10 sequences selected* GenBank Graphics Distance tree of results New MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Lasiodiplodia theobromae isolate BPPCA144 small subunit ribosomal RNA (	Lasiodiplodia the...	1002	1080	100%	0.0	99.82%	576	MK530029.1
Lasiodiplodia theobromae isolate SJS1 small subunit ribosomal RNA gene, p	Lasiodiplodia the...	1002	1073	100%	0.0	99.82%	566	OM095454.1
Lasiodiplodia theobromae isolate BPPCA167 small subunit ribosomal RNA (	Lasiodiplodia the...	998	1076	100%	0.0	99.63%	576	MK530038.1
Lasiodiplodia theobromae isolate BPPCA160 small subunit ribosomal RNA (	Lasiodiplodia the...	998	998	99%	0.0	99.82%	570	MK530033.1
Lasiodiplodia theobromae isolate BPPCA134 small subunit ribosomal RNA (	Lasiodiplodia the...	998	998	100%	0.0	99.63%	573	MK530023.1
Lasiodiplodia theobromae isolate MKMS 2.1.2 small subunit ribosomal RNA	Lasiodiplodia the...	998	998	99%	0.0	99.82%	549	MZ502166.1
Lasiodiplodia theobromae strain PaP-2 small subunit ribosomal RNA gene, p	Lasiodiplodia the...	996	996	99%	0.0	99.63%	552	MN831965.1
Lasiodiplodia theobromae strain PaS-2 small subunit ribosomal RNA gene, p	Lasiodiplodia the...	996	996	99%	0.0	99.63%	552	MN646260.1
Lasiodiplodia theobromae isolate PBBG179 small subunit ribosomal RNA ge	Lasiodiplodia the...	996	996	99%	0.0	99.63%	550	MK530048.1
Lasiodiplodia theobromae isolate BPPCA265 small subunit ribosomal RNA (	Lasiodiplodia the...	994	994	99%	0.0	99.63%	550	MK530071.1

Figure 5. Top 10 hit BLAST results against NCBI database of the sequence assembly result of the 16S rRNA PCR product of isolate K4

Figure 6. Putatively pathogenic isolate K4 subjected to antagonistic test against nystatin as control positive (left), RO water as control negative (middle), and *T. harzianum* as potential biofungicide (right). (Petri dish diameter: 8.7 cm).

ACKNOWLEDGMENT

This research was funded by the program National Flagship Program 2020-2024. The full support of Biotechnology Laboratory, National Research and Innovation Agency (BRIN), Science and Technology Park, South Tangerang, Banten, Indonesia was highly acknowledged.

STATEMENT OF AUTHORSHIP

Principal and main contributors for this study were Catur Sriherwanto, Rantika Silfarohana, Aji Wibowo, Nia Asiani, and Zhafira Amila Haqqa. Mahmud Sugianto contributed significantly to this study as well. Catur Sriherwanto: writing the English manuscript, review, editing; Rantika Silfarohana: resources, data curation, visualization, investigation, methodology; Aji Wibowo: resources, data curation, formal analysis, visualization, investigation, methodology; Zhafira Amila Haqqa: resources, data curation, visualization, investigation, methodology; Nia Asiani: resources, data curation, visualization, investigation, methodology; and Mahmud Sugianto: resources, data curation, visualization.

REFERENCES

Abtahi F, Nourani SL (2017) The most important fungal diseases associated with some useful medicinal plants. *In*: Ghorbanpour M, Varma A (eds) Medicinal Plants and Environmental Challenges. Springer, Cham, pp 279–293. doi: 10.1007/978-3-319-68717-9_16

Ali SS, Asman A, Shao J, Balidion JF, Strem MD, Puig AS, Meinhardt LW, Bailey BA (2019) Genome and transcriptome analysis of the latent pathogen *Lasiodiplodia theobromae*, an emerging threat to the cacao industry. Genome 63:37–52. doi: 10.1139/gen-2019-0112

Bhadra M, Khair A, Hossain M, Sikder M (2015) Efficacy of *Trichoderma* spp. and fungicides against *Lasiodiplodia theobromae*. Bangladesh J Sci Ind Res 49:125–130. doi: 10.3329/bjsir.v49i2.22008

Borges R, Marques E, Macedo M, Martins I, da Silva J, de Mello S (2018) Biocontrol of teak canker caused by *Lasiodiplodia theobromae*. Rev Árvore 42:e420304. doi: 10.1590/1806-90882018000300004

Chen Y, Zhang S, Lin H, Lu W, Wang H, Chen Y, Lin Y, Fan Z (2021) The role of cell wall polysaccharides disassembly in *Lasiodiplodia theobromae*-induced disease occurrence and softening of fresh longan fruit. Food Chem 351:129294. doi: 10.1016/j.foodchem.2021.129294

Degani O, Dor S (2021) Trichoderma biological control to protect sensitive maize hybrids against late wilt disease in the field. J Fungi 7:315. doi: 10.3390/JOF7040315

Dissanayak DTI, Kodituwakku TD, Kannangara B (2021) Evaluation of in vitro bio-controlling efficacy of *Trichoderma virens* against plant pathogenic fungi; *Fusarium oxysporum, Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*. In: Proceedings of the International Conference on Applied and Pure Sciences. Faculty of Science, University of Kelaniya, Sri Lanka, p 112

Erazo JG, Palacios SA, Pastor N, Giordano FD, Rovera M, Reynoso MM, Venisse JS, Torres AM (2021) Biocontrol mechanisms of *Trichoderma harzianum* ITEM 3636 against peanut brown root rot caused by *Fusarium solani* RC 386. Biol Control 164:104774. doi: 10.1016/J.BIOCONTROL.2021.104774

Huang XG, Li MY, Yan XN, Yang JS, Rao MC, Yuan XF (2021) The potential of *Trichoderma brevicompactum* for controlling root rot on *Atractylodes macrocephala*. Can J Plant Pathol 43:794–802. doi: 10.1080/07060661.2021.1933602

Intana W, Kheawleng S, Sunpapao A (2021) *Trichoderma asperellum* T76-14 released volatile organic compounds against postharvest fruit rot in muskmelons (*Cucumis melo*) caused by *Fusarium incarnatum*. J Fungi 7:46. doi: 10.3390/JOF7010046

Jiang S, Yin Q, Li D, Wu X, Wang Y, Wang D, Chen Z (2020) Integrated mRNA and small RNA sequencing for analyzing tea leaf spot pathogen *Lasiodiplodia*

theobromae, under *in vitro* conditions and the course of infection. Phytopathol 111:882–885. doi: 10.1094/PHYTO-07-20-0297-A

Juhaeti T (2002) The effect of bulb weight as planting material and shading on the growth of rodent tuber plant {*Thyponium flageliforme* (Lodd.) Bl.}. Ber Biol 6:521–526. doi: 10.14203/BERITABIOLOGI.V6I3.1227

Kamble MV, Joshi SM, Hadimani S, Jogaiah S (2021) Biopriming with rhizosphere *Trichoderma harzianum* elicit protection against grapevine downy mildew disease by triggering histopathological and biochemical defense responses. Rhizosphere 19:100398. doi: 10.1016/J.RHISPH.2021.100398

Kamil FH, Saeed EE, El-Tarabily KA, Abu Qamar SF (2018) Biological control of mango dieback disease caused by *Lasiodiplodia theobromae* using Streptomycete and non-Streptomycete actinobacteria in the United Arab Emirates. Front Microbiol 9:829. doi: 10.3389/fmicb.2018.00829

Khalivulla SI, Mohammed A, Sirajudeen KNS, Shaik MI, Ye W, Korivi M (2019) Novel phytochemical constituents and anticancer activities of the genus, *Typhonium*. Curr Drug Metab 20:946–957. doi: 10.2174/1389200220666191118102616

Kumar S, Chandra R, Behera L, Keswani C, Sansinenea E (2021) Dual *Trichoderma* consortium mediated elevation of systemic defense response against early blight in potato. Eur J Plant Pathol 2021 1–16. doi: 10.1007/S10658-021-02431-4

Kushwaha RK, Singh S, Pandey SS, Rao DKV, Nagegowda DA, Kalra A, Vivek Babu CS (2019) Compatibility of inherent fungal endophytes of *Withania somnifera* with *Trichoderma viride* and its impact on plant growth and withanolide content. J Plant Growth Regul 38:1228–1242. doi: 10.1007/S00344-019-09928-7

Kwak Y (2021) An update on *Trichoderma* mitogenomes: Complete *de novo* mitochondrial genome of the fungal biocontrol agent *Trichoderma harzianum* (Hypocreales, Sordariomycetes), an ex-neotype Strain CBS 226.95, and tracing the evolutionary divergences of mitogenomes in *Trichoderma*. Microorg 9:1564. doi: 10.3390/MICROORGANISMS9081564

Liu HJ, Duan WD, Liu C, Meng LX, Li HX, Li R, Shen QR (2021) Spore production in the solid-state fermentation of stevia residue by *Trichoderma guizhouense* and its effects on corn growth. J Integr Agric 20:1147–1156. doi: 10.1016/S2095-3119(20)63478-5

Manganiello G, Nicastro N, Caputo M, Zaccardelli M, Cardi T, Pane C (2021) Functional hyperspectral imaging by high-related vegetation indices to track the wide-spectrum *Trichoderma* biocontrol activity against soil-borne diseases of baby-leaf vegetables. Front Plant Sci 12: 630059. doi: 10.3389/FPLS.2021.630059

Meher J, Rajput RS, Bajpai R, Teli B, Sarma BK (2020) *Trichoderma*: A globally dominant commercial biofungicide. In: Manoharachary C, Singh HB, Varma A (eds) Trichoderma: Agricultural Applications and Beyond, Springer International Publishing, Cham, pp 195–208. doi: 10.1007/978-3-030-54758-5_9

Moreira S, da Consolacao Dutra D, Rodrigues A, de Oliveira J, Dhingra O, Pereira O (2013) Fungi and bacteria associated with post-harvest rot of ginger rhizomes in Espírito Santo, Brazil. Trop Plant Pathol 38:218–226. doi: 10.1590/S1982-56762013000300006

Mousumi Das M, Aguilar CN, Haridas M, Sabu A (2021) Production of bio-fungicide, *Trichoderma harzianum* CH1 under solid-state fermentation using coffee husk. Bioresour Technol Reports 15:100708. doi: 10.1016/J.BITEB.2021.100708

Oszako T, Voitka D, Stocki M, Stocka N, Nowakowska JA, Linkiewicz A, Hsiang T, Lassaad B, Berezovska D, Malewski T (2021) *Trichoderma asperellum* efficiently protects *Quercus robur* leaves against *Erysiphe alphitoides*. Eur J Plant Pathol 159:295–308. doi: 10.1007/S10658-020-02162-Y/FIGURES/3

Pandi M, Kumaran RS, Choi YK, Kim HJ, Muthumary J (2011) Isolation and

detection of taxol, an anticancer drug produced from *Lasiodiplodia theobromae*, an endophytic fungus of the medicinal plant *Morinda citrifolia*. Afr J Biotechnol 10:1428–1435

Ruangwong OU, Pornsuriya C, Pitija K, Sunpapao A (2021) Biocontrol mechanisms of *Trichoderma koningiopsis* PSU3-2 against postharvest anthracnose of chili pepper. J Fungi 7:276. doi: 10.3390/JOF7040276

Rusin C, Cavalcanti F, de Lima PCG, Faria CMD, Almança MAK, Botelho RV (2021) Control of the fungi *Lasiodiplodia theobromae*, the causal agent of dieback, in cv. syrah grapevines. Acta Sci Agron 43:e44785. doi: 10.4025/actasciagron.v43i1.44785

Sala A, Vittone S, Barrena R, Sánchez A, Artola A (2021) Scanning agro-industrial wastes as substrates for fungal biopesticide production: Use of *Beauveria bassiana* and *Trichoderma harzianum* in solid-state fermentation. J Environ Manage 295:113113. doi: 10.1016/J.JENVMAN.2021.113113

Santos PHD, Carvalho BM, Aredes FAS, Mussi-Dias V, Pinho DB, Pereira MG, da Silveira SF (2020) Is *Lasiodiplodia theobromae* the only species that causes leaf blight disease in Brazilian coconut palms? Trop Plant Pathol 45:434–442. doi: 10.1007/s40858-020-00344-x

Septaningsih DA, Yunita A, Putra CA, Suparto IH, Achmadi SS, Heryanto R, Rafi M (2021) Phenolics profiling and free radical scavenging activity of *Annona muricata*, *Gynura procumbens*, and *Typhonium flagelliforme* leaves extract. Indones J Chem 21:1140–1147. doi: 10.22146/IJC.62124

Singh A, Pandey R (2020) Management of diseases of medicinal and aromatic plants using high shelf life formulation of *Trichoderma*. In: Manoharachary C, Singh HB, Varma A (eds) Trichoderma: Agricultural Applications and Beyond. Springer International Publishing, Cham, pp 181–194. doi: 10.1007/978-3-030-54758-5_8

Vinayarani G, Madhusudhan KN, Prakash HS (2019) Induction of systemic resistance in turmeric by rhizospheric isolate *Trichoderma asperellum* against rhizome rot disease. J Plant Pathol 101:965–980. doi: 10.1007/S42161-019-00303-9

Wanjiku EK, Waceke JW, Mbaka JN (2021) Suppression of stem-end rot on avocado fruit using *Trichoderma* spp. in the central highlands of Kenya. Adv Agric 2021:8867858. doi: 10.1155/2021/8867858

Webber TV, Martins TV, Cândida DV, Reis CAF, da Cunha MG, Sette Jr CR, de Campos Dianese É (2021) Control techniques and evaluation of pathogen influence on African mahogany (*Khaya grandifoliola* C. Dc.) infected by *Lasiodiplodia theobromae* Pat. Eur J Plant Pathol 159:427–432. doi: 10.1007/s10658-020-02153-z

Yu Z, Wang Z, Zhang Y, Wang Y, Liu Z (2021) Biocontrol and growth-promoting effect of *Trichoderma asperellum* TaspHu1 isolate from *Juglans mandshurica* rhizosphere soil. Microbiol Res 242:126596. doi: 10.1016/J.MICRES.2020.126596

Zhang C, Wang W, Xue M, Liu Z, Zhang Q, Hou J, Xing M, Wang R, Liu T (2021) The combination of a biocontrol agent *Trichoderma asperellum* SC012 and hymexazol reduces the effective fungicide dose to control fusarium wilt in cowpea. J Fungi 7:685. doi: 10.3390/JOF7090685

Zhang W, Yan J, Li X, Xing Q, Chethana KWT, Zhao W (2019) Transcriptional response of grapevine to infection with the fungal pathogen *Lasiodiplodia theobromae*. Sci Rep 9:5387. doi: 10.1038/s41598-019-41796-9

Zheng Q, Ozbudak E, Liu G, Hosmani PS, Saha S, Flores-Gonzalez M, Mueller LA, Rodrigues-Stuart K, Dewdney MM, Lin Y, Zhang J, Tarazona YC, Liu B, Oliva R, Ritenour MA, Cano LM (2020) Draft genome sequence resource of the citrus stem-end rot fungal pathogen *Lasiodiplodia theobromae* CITRA15. Phytopathol 111:761–764. doi: 10.1094/PHYTO-08-20-0349-A