Journal of Tropical Biodiversity and Biotechnology
Volume 08, Issue 01 (2023): jtbb76897
DOI: 10.22146/jtbb.76897

Short Communication

Diversity of *Fusarium* Endophytes Isolated from Wild Bananas in Pandenglang, Indonesia

Nani Maryani[1,2]*, Siti Yuliyanti[1], Indriati Ramadhani[3], Rida Oktorida Khastini[1], Suroso Mukti Leksono[4]

1)Biology Education, Universitas Sultan Ageng Tirtayasa (UNTIRTA), Jl. Raya Ciwaru No.25, Kota Serang, Banten 42124
2)Center of Excellence for Local Food Innovation, Jl. Raya Palka Km.3 Sindangsari, Kec. Pabuaran, Serang, Banten 42163
3)Research Center for Biology, National Research and Innovation Agency (BRIN), Cibinong Bogor
4)Science Education, Universitas Sultan Ageng Tirtayasa (UNTIRTA), Jl. Raya Ciwaru No.25, Kota Serang, Banten 42124
* Corresponding author, email: nani.maryani@untirta.ac.id

Keywords:
Banten
Endophyte
fungi
Fusarium
Musa
Submitted:
05 August 2022
Accepted:
16 January 2023
Published:
03 March 2023
Editor:
Furzani Binti Pa'ee

ABSTRACT

A group of *Fusarium* spp., in the *Fusarium oxysporum* species complex is known as pathogens on bananas, i.e., Fusarium wilt or Panama Disease. However, many *Fusarium* spp. are also known to be endophytes inside healthy banana plants and have been less explored and investigated. *Fusarium* endophytes have been demonstrated to be effective against the *Fusarium* pathogen that causes wilting in some crops such as tomatoes and watermelon. Thus, we explored endophytes *Fusarium* from local bananas in Pandenglang Banten for further use as biocontrol of Fusarium wilt. Four wild banana accessions were identified, from which 9 *Fusarium* isolates recovered from its pseudostems asymptomatic plants. All isolates were characterized based on their morphological characters and sequence of the Internal Transcribed Spacer (ITS) gene. These isolates belong to four complexes of *Fusarium* i.e. *Fusarium equiseti* species complex, *Fusarium oxysporum* species complex, *Fusarium sambucinum* species complex, and *Fusarium solani* species complex (currently described as *Neocosmospora*). Further study on molecular characterization of these isolates using specific genes and their potential antagonists of pathogens still needs to be discovered for other use as a biocontrol against Fusarium wilt.

Banana is one of the fruit commodities that plant pathologies have given many intentions for the past two decades because of the emerging destructive disease called Fusarium wilt, known as Panama Disease (Ploetz 2006; Ordonez et al. 2015; Westerhoven et al. 2022). However, in a country where banana is native such as Indonesia, the awareness of the disease lacks of attention. Moreover, the abundant diversity and luxury of choosing many different bananas to the table make the condition unheard for most people. The unawareness of farmers to isolate their banana plants that are infected with Fusarium wilt perhaps cause the pathogen, *Fusarium* spp., evolve with the host with different varieties of banana (Ploetz et al. 1999; O'donneal et al. 2008; Maryani 2018).

Fusarium wilt on a banana can be recognized by symptoms such as wilting and yellowing of the leaves, longitudinal splitting of the pseudostem, internal pseudostem showing reddish and brownish vascular lines (Maryani 2018). Once banana plants are infected with Fusarium

wilt, the soil becomes contaminated with the pathogen that can be survived for decades. This is because the chlamydospores of *Fusarium* spp. can survive more than 30 years in soil (Ploetz 2006). To date, there is no effective strategy to control the Fusarium wilt of bananas except to exclude the pathogen from non-infested areas, prevention by using pathogen-free tissue culture plants, and quarantine strategies (Kema et al. 2021). Applying disinfectant against soil pathogens could harm the microbial community in the soil, anaerobic soil disinfestation might be too costly, and the development of resistant cultivars is not foreseen in the short-term (Dale et al. 2017; Salacinas 2019; Ahmad et al. 2020). Therefore, biological control is considered a promising alternative to manage the disease and is environmentally friendly.

Endophytes are microbes that colonize plant tissue without causing disease. Many endophytes microorganism is known to decrease the disease susceptibility of their host upon infection. Thus, it is an attractive agent for organic farming. In the case of fungal *Fusarium* spp., despite its abundant species as a pathogen of many important crops, many of these species are endophytes (Leslie & Summerell 2006). Some *Fusarium* endophytes can protect the plants against Fusarium wilt caused by pathogenic *Fusarium* (Aimé et al. 2013). *Fusarium oxysporum* endophyte isolates Fo47 is well studied and known for reducing Fusarium wilt in many crops, including tomato, asparagus, cotton, and chickpea (Zhang et al. 2018; Constantine et al. 2020). However, the potential of fungal endophytes against Fusarium wilt on a banana is unknown. Therefore, studying *Fusarium* endophytes to fight the Fusarium wilt of bananas is crucial and will give insights into the possibility of managing this disease in a friendly-environmentally way. As a first step towards that goal, this study aimed to identify endophytic fungi from wild banana (*Musa* spp.) in Kabupaten Pandeglang Banten Province, Indonesia, for later use as a biocontrol against Fusarium wilt on banana.

Sampling collection was undertaken through exploration in six districts of Pandeglang Banten; Cadasari, Banjar, Menes, Cisata, Saketi, and Pandeglang. Wild banana was able to be identified based on the presence of abundant seed in their fruit (Figure 1). Pseudostem from symptomless plants was sampled and placed on filter paper to dry and packed in a paper envelope. Global Positioning System (GPS) coordinates were recorded and ecological parameters including soil pH, light intensity and vegetation around the sampling area, were noted at each site. Wild banana identification was based on morphological characters (Valmayor et al. 1999) and in-situ comparison with *Musa* collection at Kebun Plasma Nutfah, Pusat Biologi, Cibinong, Bogor, Indonesia (Poerba et al. 2018).

Samples were isolated using the direct plating method with surface sterilization (Maryani 2018). Dried pseudostem was cut into pieces, approximately 2 x 3 cm, and plated into potato dextrose agar (PDA) plates. After 2-3 days, colonies resembling fungi were transferred to new PDA plates. Monosporic culture was derived by streaking small amount of conidia, collected with the tip of inoculation needle on water agar (WA) plates, which allowed conidia to separate. After 24h incubation, plates were observed under stereo microscope and germinating conidia were collected and transferred to new PDA. Monosporic isolates were maintained on PDA for working culture and 20% (v/v) glycerol at -20^{0}C for long preservation. All isolates were deposited at the laboratory of Biology Education Culture Collection, Universitas Sultan Ageng Tirtayasa (UNTIRTA) Banten.

Fungal isolates were grown on carnation leaves agar (CLA) for sporodochium formation, synthetic nutrient agar (SNA) for chlamydo-

spore formation, and PDA for conidia formation. All cultures on each medium were incubated under continuous light at room temperature. Growth rates of isolates were determined on PDA plates, after 7-day incubation at room temperature in the dark. All isolates were identified based on their morphological characters (Seifert et al. 2011; Maryani 2018) as well as comparison with well-identified living culture from the collection of Biology Education Laboratory, UNTIRTA and Typed isolates from Indonesian Culture Collection (InaCC) BRIN, Cibinong. Molecular identification of the isolates was using pair of primers ITS4 (5`-TCC TCC GCT TAT TGA TAT GC- 3`) and ITS5 (5`-GGA AGT AAA AGT CGT AAC AAG G-3`) which amplified the ITS (Internal Transcribed Spacer) regions (White et al. 1990). Fungal DNA extraction following the protocol of Hidayat & Ramadhani (2019).

Both ancestors of cultivated bananas, *Musa acuminata* Colla dan *Musa balbisiana* Colla were found in this exploration (Nasution 1990). *M. acuminata* and *M. balbisiana* can be distinguished by their male flower, leaf petiole, and fruit (Ahmad 2021). The flower of *M. acumianata* has no pigmentation, usually white or creamy. In contrast, the flower of *M. balbisiana* has red or red-to-purple pigmentation. Opened brachtea of the male bud of *M. acuminata* is rolled up, but not for *M. balbisiana. M. acuminata* has opened petiole canal leaf, but the petiole canal leaf of *M. balbisiana* is closed or inward. The transverse of the fruit of *M. acuminata* is rounded, while that of *M. balbisiana* is pronounced ridges.

None of the wild bananas showed any symptoms of diseases. Leaves diseases commonly identified on cultivated bananas were absent, and pseudostem was clean from any internal symptoms of wilt disease of bananas. All identified wild bananas are seeded, less pulpous, and found in abandoned areas or near rivers (Figure 1). Three wild banana species were found in four locations at *Kabupaten Pandenglang*, of which only one of *Musa* species could be identified in variety levels, *Musa acuminata* var. *breviformis* (Table 1).

In total, 9 *Fusarium* isolates were recovered from the asymptomatic pseudostem of *M. acuminata* and *M. balbisiana* (Table 2). *Musa acuminata* identified beside a river at Desa Cisata Pandegang bared the highest number and diversity of *Fusarium. M. balbisiana* species from Saketi obtained only one isolate of *Fusarium* sp. The rest of the wild *Musa* discovered in this study were not contained *Fusarium* isolates in their pseudostem. Combining morphology and molecular identification using Internal Transcribed spacer (ITS) gene (Supplementing Table 1.), we can identify four complex members of *Fusarium* i.e., *Fusarium equiseti species complex* (Figure 2), *F. oxysporum species complex* (Figure 3), *F. sambucinum species complex* (Figure 4), *and F. solani species complex* (Figure 5).

Figure 1. Wild bananas collected in Pandeglang. A-B. *Musa acuminata*, C. *M. balbisiana.*

Table 1. Details Location, GPS, and Wild *Musa* Species Found in Pandeglang as a Source of Isolation Endophytic Fungi.

Location		GPS			Wild Musa	Genome
District	Village	Long.	Lat.	Alt. (m)		
Menes	Tegalwangi/ Kampung Cipancur	105.93	-6.39	34,5	*Musa acuminata*	AA
Cisata	Cisata	106.09	-6.32	59.4	*Musa acuminata*	AA
Pandeglang	Cikondang	105.93	-6.39	231	*M. acuminata* var. *breviformis*	AA
Saketi	Kadu Dampir	105.96	-6.39	129	*Musa. balbisiana*	BB

Table 2. List of the diversity of *Fusarium* isolates recovered from pseudostem of wild banana, based on morphology and molecular identification on ITS gene sequence.

Isolate code	Species name	*Fusarium* complexes	Host	Location
NMC273	*Fusarium* sp.	NA		
NMC274	*Fusarium solani*	*Fusarium solani species complex*		
NMC276	*Fusarium oxysporum*	*Fusarium oxysporum species complex*		
NMC277	*Fusarium solani*	*Fusarium solani species complex*		
NMC278	*Fusarium equiseti*	*Fusarium incarnatum-equiseti complex*	*M. acuminata*	Cisata
NMC279	*Fusarium longipes*	*Fusarium sambucinum species complex*		
NMC280	*Fusarium solani*	*Fusarium solani species complex*		
NMC281	*Fusarium* sp.	NA		
NMC293	*Fusarium* sp.	NA	*M. balbisiana*	Saketi

It is well-known, reported and studied that the primary pathogen of bananas is *Fusarium*. More in this research, *Fusarium* is categorized as endophytic fungi. Symptomless infections by *Fusarium* species have been reported in many plants, such as medicinal plants (Jia et al. 2016) and agricultural plants (Zakaria & Ning 2013; Nuraini et al. 2017). In bananas, *Fusarium* is reported to recover from symptomatic and asymptomatic plants with Fusarium wilt diseased (Maryani 2018; Maryani et al. 2019). *Fusarium oxysporum* was reported to recover from the roots of wild *Musa acuminata* from Malaysia but not in their pseudostem (Zakaria et al. 2011). Interestingly, the most devastated pathogen of bananas, Tropical Race 4, is also a member of the *Fusarium oxysporum* species complex (Ordonez et al. 2015; Maryani 2018). Comparative studies between these members of the complex will be beneficial in revealing why wild bananas are rarely found to be infected with fusarium wilt.

Fusarium equiseti is the most reported species isolated from each part of the banana plants, including leaves, fruit, roots, and pseudostem (Zheng et al. 2012; Zakaria & Wan Aziz 2018; Maryani et al. 2019). *Fusarium longipes* is never reported from the pseudostem of asymptomatic bananas since its first description by Reinking & Wollenweber (1927), who isolated from leaves *Musa sapientum*. *Fusarium solani*, currently recognized as a member of the genus *Neocosmospora*, is known to be an opportunistic pathogen on plants and animals (Sandoval et al. 2019). Thus, it is interesting that most of the isolates we discover in this study are members of this complex.

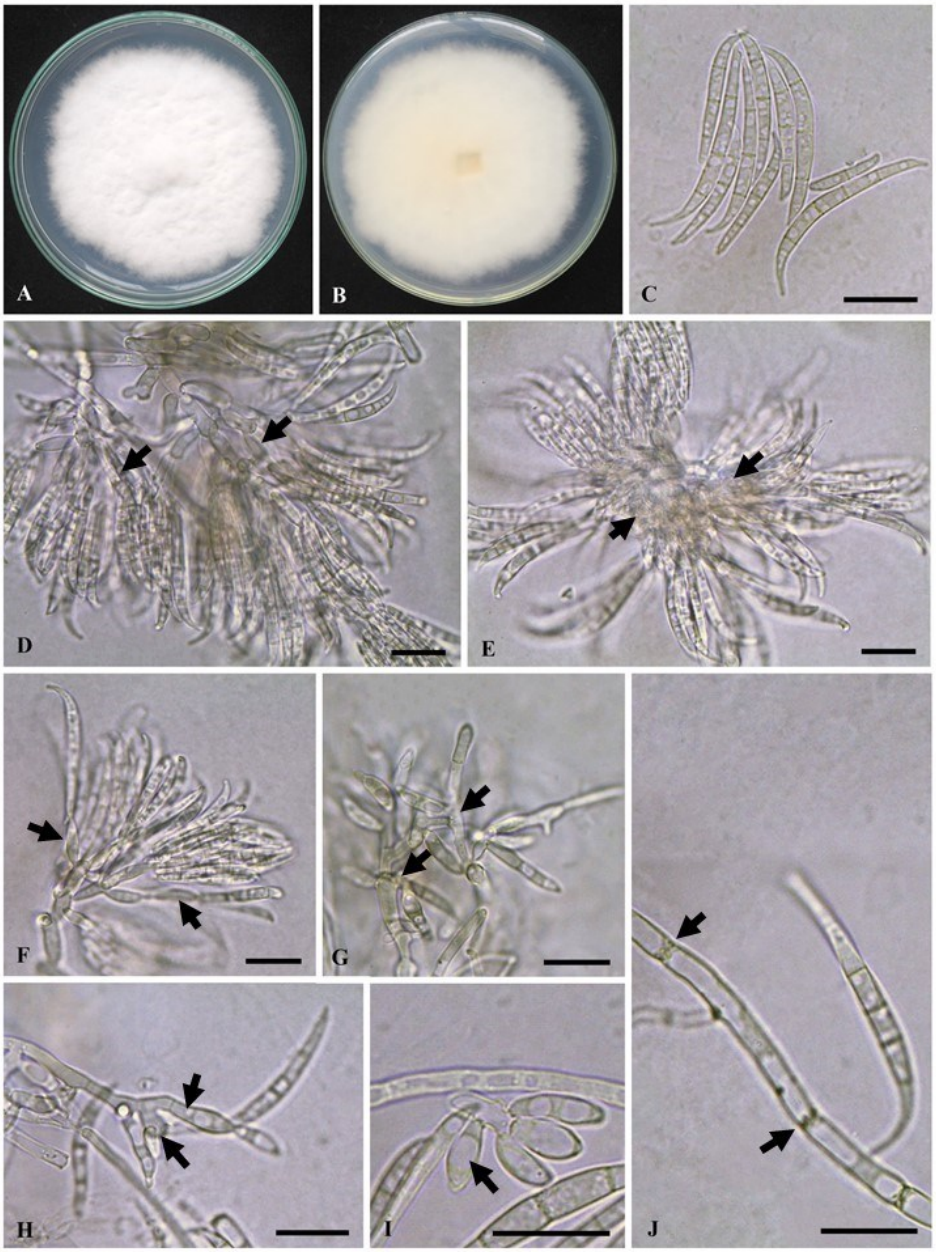

Figure 2. *Fusarium equiseti* NMC278. **A-B**. Culture grown on PDA after 7d incubation, **C**. Macroconidia, **D-G**. Aerial phialides, **H**. Polyphialid, **I**. Conidiogenous cell, **J**. Hyphae septate. Scale bars C-J = 10 µm.

The role of the *Fusarium* endophyte inside the banana plants is still to be discovered. However, some studies demonstrated that *Fusarium* could decrease disease incidence in some crops with wilt disease (Aimé et al. 2013; Zhang et al. 2018; Constantine et al. 2020). Future studies on the potential of *Fusarium* endophyte to control primary disease on bananas, i.e., Fusarium wilt, will be critical as both types of *Fusarium* colonies have the same host. Moreover, endophyte *Fusarium* isolated from the wild banana will be interesting as many wild bananas are resistant to Fusarium wilt (Ahmad et al. 2020). In this study, we expand the knowledge of the diversity of endophytic fungi from wild bananas. Further characterization of the isolates based on molecular data using specific genes in each genus is needed. The role of each of these fungi inside healthy bananas and their potential to be antagonists of pathogens are also to be discovered for further use as a biocontrol against Fusarium wilt.

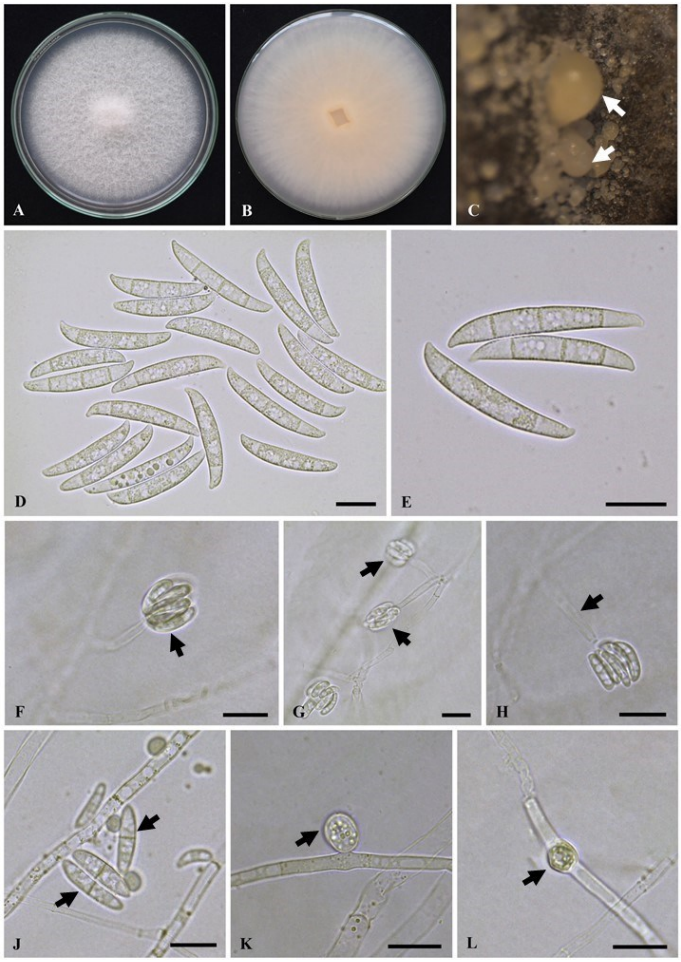

Figure 3. *Fusarium oxysporum* NMC276, **A-B.** Culture grown on PDA after 7d incubation, **C.** Sporodochia on CLA, **D-E.** Macroconidia, **F-G.** False head, **H.** Monophialide, **J.** Microconidia, **K-L.** Chlamydosphore. Scale Bares C-L = 10 µm.

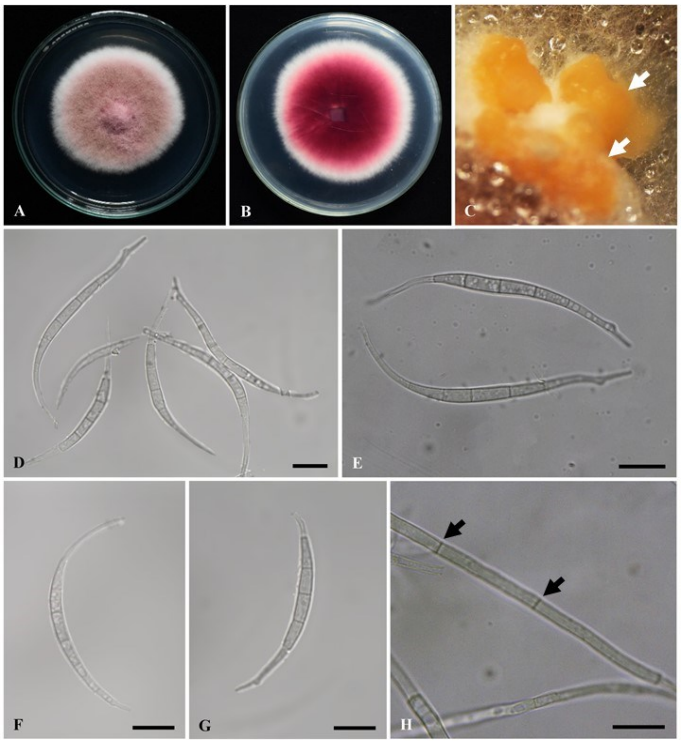

Figure 4. *Fusarium longipes* NMC 279 member of *Fusarium sambucinum* species complex. A-B. Culture grown on PDA after 7d incubation, C. Sporodochia on CLA, D-G. Macroconidia, H. Hyphae septate. Scale bars C-H= 10µm.

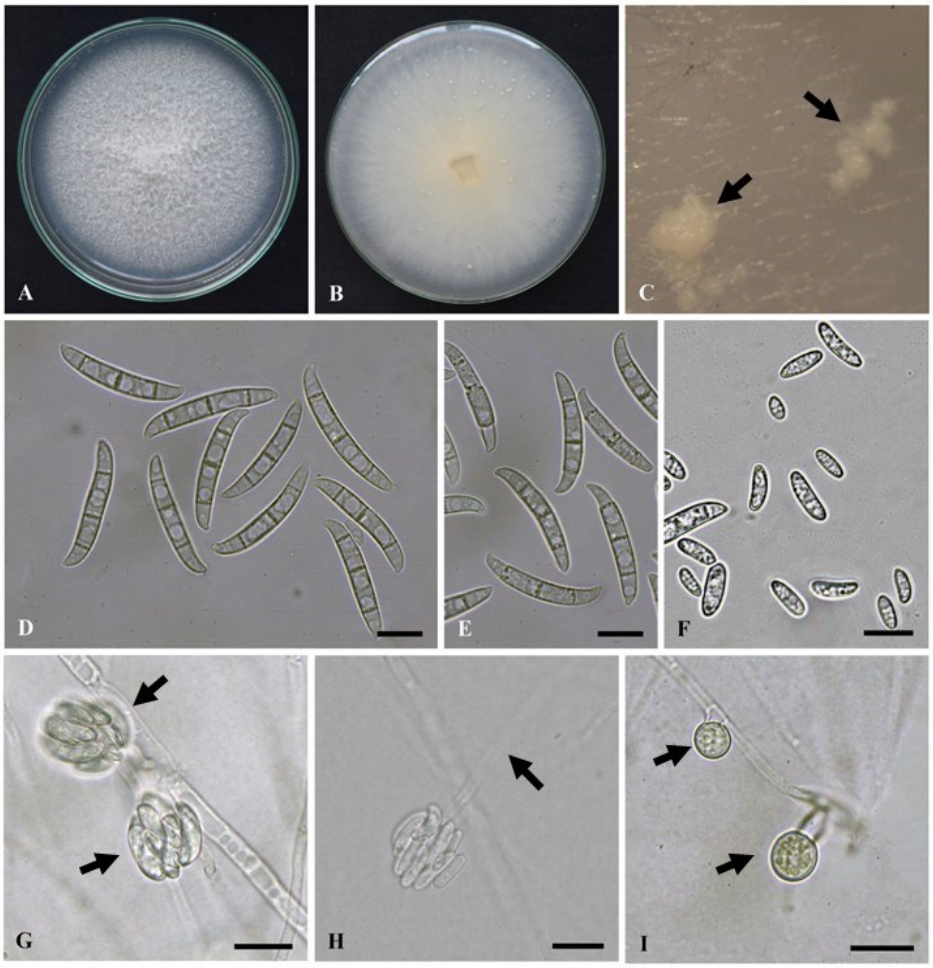

Figure 5. *Fusarium solani* NMC277. **A-B.** Culture grown on PDA after 7d incubation, **C.** Sporodochia on CLA, **D-E** Macroconidia, **F.** Microconidia, **G.** False head, **H**. Monophialid, **I.** Chlamydosphore. Scale bars C-I = 10 µm.

AUTHOR CONTRIBUTION

NM designed the research, collected and analysed the data, and wrote the manuscript, SY executed the research in the laboratory, IR sequenced *Fusarium* isolates, ROK and SML supervised SY.

ACKNOWLEDGMENTS

We thank Dr. Fajarudin Ahmad, Research Center for Genetic Engineering-BRIN for joining us in the collection and helping us for wild *Musa* identification.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the research or the research funding.

REFERENCES

Ahmad, F., Maryani, N. & Poerba, Y.S., 2020. Genetic mapping of Fusarium wilt resistance in a wild 1 banana *Musa acuminata* ssp. *malaccensis* accession. *TheorApplyGenet.*, 133, pp.3409-3418. doi: 10.1007/s00122-020-03677-y.

Ahmad, F., 2021. *Genetics and diversity of Indonesian Bananas.* Wageningen University and Research (WUR): The Netherlands.

Aimé, S. et al., 2013. The endophytic strain *Fusarium oxysporum* Fo47: A good candidate for priming the defense responses in tomato roots. *Mol Plant-Microbe Interact.*, 26 (8), pp.918–926. doi: 10.1094/MPMI-12-12-0290-R.

Constantine, M.E., Vlieger, B.V. & Takken, F.L.W., 2020. Diminished Pathogen and Enhanced Endophyte Colonization upon CoInoculation of Endophytic and Pathogenic *Fusarium* Strains. *Microorganisms*, 8(4), pp.544. doi: 10.3390/microorganisms8040544.

Dale, J., James, A. & Paul, J.Y., 2017. Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4. *Nat Commun.*, 8, Article number: 1496. doi: 10.1038/s41467-017-01670-6

Hidayat, I. & Ramadhani, I., 2019. Phylogenetic study of Curvularia on Sorgum from Indonesia Based on ITS rDNA Sequence. *Jurnal Mikologi Indonesia*, 3(2), pp.118-124.

Jia, M. et al., 2016. A Friendly Relationship between Endophytic Fungi and Medicinal Plants: A Systematic Review. *Front Microbiol*, 7, pp.906. doi: 10.3389/fmicb.2016.00906.

Kema, G.H.J., et al. 2021. Editorial: Fusarium Wilt of Banana, a Recurring Threat to Global Banana Production. *Front. Plant Sci.*, 11, 628888. doi: 10.3389/fpls.2020.628888

Leslie, J.F. & Summerell, B.A., 2006. *The Fusarium laboratory manual*, UK: Blackwell Publishing Ltd.

Maryani, N., 2018. *A complex relationship: Banana and Fusarium wilt in Indonesia.* Wegeningen University and Research (WUR): The Netherlands.

Maryani, N. et al., 2019. New Endemic Fusarium species hitch-hiking with Pathogenic Fusarium Strains Causing Panama disease in small -holder Banana Plots in Indonesia. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, 43, pp.48-69. doi: 10.3767/ persoonia.2019.43.02

Nasution, R.E, 1990. *A taxonomic Study of the Species Musa acuminata Colla with its intraspecific taxa in Indonesia.* Tokyo University of Agriculture, Japan.

Núñez-Trujillo, G. et al., 2013. Survey of Banana endophytic fungi isolated in artificial culture media from an applied viewpoint. *J Horti Fores Biotech*, 17, pp.22-25.

Nuraini, F.R., Setyaningsih, R. & Susiolowati, A., 2017. Screening and Characterization of Endophytic Fungi as Antagonistic Agents Toward *Fusarium oxysporum* on Eggplant (*Solanum melongena*). *Biodiversitas*, 18(4), pp.1377-1384. doi: 10.13057/biodiv/d180413

O'donneal, K. et al., 2008. Molecular Phylogenetic Diversity, Multilocus Haplotype Nomenclature, and in vitro Antifungal Resistance within the *Fusarium solani* species Complex. *J Clin Microbiol*, 46(8), pp.2477-2490. doi: 10.1128/JCM.02371-07.

Ordonez, N. et al., 2015. Worse comes to worst: banana and Panama disease: when plant and pathogen clones meet. *PLoS Pathogen*, 11(11), e1100519. doi: 10.1371/journal.ppat.1005197.

Ploetz, R.C., Haynezm, J., & Vazquez, A., 1999. Responses of New Banana Accessions in South Florida to Panama Disease. *Crop Prot*, 18, pp.445-449. doi: 10.1016/S0261-2194(99)00043-5

Ploetz, R.C., 2006. Panama disease, an old nemesisrears its ugly head: part2, the Cavendish era and beyond. *Plant Health Progress*, 7(1), pp.1-17. doi: 10.1094/PHP-2006-0308-01-RV

Poerba, Y.S., Witjaksono, D. & Martanti, 2018. *Katalog Pisang: Koleksi Kebun Plasma Nutfah Pisang Pusat Penelitian Biologi Lembaga Ilmu Pengetahuan Indonesia*, Jakarta: LIPI Press.

Reinking, A.O. & Wollenweber, H.W., 1927. Tropical Fusaria. *The Philippine Journal of Science*, 32(2), pp.104–244.

Salacinas, M., 2019. *Spot On: Managing Panama Disease of Banana in The Philippines.* Wegeningen University: The Netherlands.

Sandoval-Denis, M., Lombard, L. & Crous, P.W., 2019. Back to the roots: a reappraisal of Neocosmospora. *Persoonia*, 43(96), pp.90-185. doi: 10.3767/persoonia.2019.43.04

Seifert, K. et al., 2011. *The Genera of Hyphomycetes, CBS biodiversity series No.9.* Utrecht, The Netherlands: CBS-KNAW Fungal Biodiversity Center.

Valmayor, R., Jamaluddin, S.H. & Silahoy, J.S.B., 1999. *Banana cultivar names and synonyms in Southeast Asia*, INIBAP Montpeliier France.

Westerhoven, A.C.V., et al. 2022. Dissemination of Fusarium wilt of banana in Mozambique caused by *Fusarium odoratissimum* Tropical Race 4. Plant *Disease*. doi: 10.1094/PDIS-07-22-1576-SC.

White, T.J. et al., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: A guide to methods and applications*. New York: Academic Press, pp.315–322.

Zakaria, L. & Rahman, N.H.A., 2011. Endophytic *Fusarium* spp. from wild banana (*Musa acuminata*) roots. *African Journal of Microbiology Research*, 5(21), pp.3600-3602.

Zakaria, L. & Ning, C.H., 2013. Endophytic *Fusarium* spp. from Roots of Lawn Grass (*Axonopus compressus*). *Trop Life sci Res.*, 24(2), pp.85–90. PMCID: PMC3935371

Zakaria, L. & Wan Aziz, W.N., 2018. Molecular identification of Endophytic Fungi from Banana Leaves (Musa spp.). *Trop Life sci Res.*, 29 (2), pp.201–211. doi: 10.21315/tlsr2018.29.2.14

Zhang, J., Chen, J. & Jia, R.M., 2018. Suppression of plant wilt diseases by nonpathogenic *Fusarium oxysporum* Fo47 combined with actinomycete strains. *Biocontrol Sci Technol.*, 28(6), pp.562– 573. doi: 10.1080/09583157.2018.1468996.

Zheng, L.S.et al. 2012. The *Fusarium* species isolated from banana and their phylogenetic relationships. *Mycosystema*, 32(4), 617–632.