

ORIGINAL ARTICLE

Diversity of the fungal community on mango associated with stem end rot and anthracnose diseases based on amplicon targeted metagenomics

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Abstract

This study aimed to comprehend the diversity of the fungal community on Chokanan mango, a premium mango variety from Thailand which is widely cultivated in Indonesia, associated with stem end rot and anthracnose disease using high-throughput amplicon targeted metagenomics analysis by next-generation sequencing (NGS). Samples used in this study were freshly harvested healthy fruits at the age of 15-weeks (H15.ITS), healthy fruits after 2 weeks incubation (H17.ITS), 17-week old fruits (S17.ITS) with stem end rot symptoms, and 17-week old fruits (A17.ITS) with anthracnose symptoms. Results showed that the Basidiomycota phylum was dominant in the healthy fruits, while the Ascomycota phylum was found dominantly in sick fruits. Based on OTUs alignment of sequenced data, some species found to be dominantly associated with stem end rot disease in this study were *Lasiodiplodia theobromae*, *Neofusicoccum cordaticola* and *N. mangiferae*. Dominant species which were associated with mango anthracnose disease were *Colletotrichum gloeosporioides*, *Botryosphaeria corticis*, *Volutella* sp., and *Pseudofusicoccum violacearum*. These fungal genera were not found to be dominant in healthy fruits at the same age indicating that specific genera contributed to developing postharvest diseases on mango differently. The findings confirmed that the fungal community associated with stem end rot and anthracnose disease on mango was unique, and specific species contributed in particular disease development. Since mango is an important global commodity, these research findings will contribute significantly to global biosecurity.

Keywords: *Lasiodiplodia theobromae*, microbiome interaction, *Neofusicoccum cordaticola*, *Neofusicoccum mangiferae*, postharvest disease

Introduction

Mango (*Mangifera indica* L.), the “king of fruit”, is one of Indonesia’s most important fruit commodities because it has an exotic appeal and high economic value both domestically and for export. Total production in 2022 was 3.3 million tons, which was an increase from the previous year by as much as 2.8 million tons (Statistics Indonesia 2023). This year, mango export volume grew to 939 tons with a selling price of USD 994.3 per ton (Puspitasari 2023). Indonesia, which is acknowledged as the world’s fourth-largest mango producer, faces the urgent challenge of

meeting the high standards set by customers (Kiloes *et al.* 2023).

Chokanan, one of high-quality mangos from Thailand, has a unique color, aroma, and flavor. Recently it is being cultivated in Indonesia in response to the high demand of domestic consumers. It has an oval form, is medium in size with bright yellow skin and orange-yellow flesh, with a sweet taste (TSS: 14–16%). It is also pleasant-smelling, slightly fibrous and has a high production of approximately 60–100 fruits per tree (Azhar *et al.* 2013). The flowers bloom all year round,

therefore it has the potential to address issues on global food availability.

Stem end rot and anthracnose disease are two top postharvest diseases on mango worldwide including Indonesia (Galsurker *et al.* 2018; Lu *et al.* 2022; Widiastuti *et al.* 2023). These diseases are difficult to control since they appear on ripe fruit, generally when the fruit reaches the consumer or the retailer, therefore producers often receive complaints. Potential yield loss due to stem end rot disease ranged from 30–40% (Galsurker *et al.* 2018), while losses caused by anthracnose disease reached 60–100% (Uddin *et al.* 2018; Benatar *et al.* 2021). These two diseases are presently the biggest constraint of Indonesian export of mango fruits. Due to the development of advanced methods for monitoring pathogens and diagnosis tools, recent studies reveal that pathogens causing disease are usually not only single species but are a consortium or complex of pathogen species (Li *et al.* 2019; Galsurker *et al.* 2018). Galsurker *et al.* (2018) stated that stem end rot disease on mango caused by Botryosphaeriaceae fungi were: *Dothiorella dominicana*, *D. mangiferae*, *L. theobromae*, *Neofusicoccum* spp., also *Phomopsis mangiferae*, *Cytosphaera mangiferae*, *Pestalotiopsis* sp., *Colletotrichum gloeosporioides* and *Alternaria alternata*. They were a group of fungi that initially lived as natural inhabitants on mango branches, and grew at the bottom of fruit or stem ends as endophytic microbes. They then started their necrotrophic phase along with fruit ripeness, and ultimately caused symptoms of fruit rot (Galsurker *et al.* 2020). Therefore, understanding the dynamics of microbial communities during fruit development is crucial to comprehending the pathogen – host interaction, the pathogenesis process and management of postharvest diseases. Moreover, Widiastuti *et al.* (2023) reported that both diseases could infect fruit together at the same time and both disease symptoms were also obviously seen. To date, no research has compared how microbial communities affected the development of each disease. Considering the above evaluation, this research was designed to evaluate if a specific community was involved in each particular disease.

A metagenomic approach has significantly contributed to determining the biodiversity of the microbiome regulating disease development (Pinto *et al.* 2014; Taylor *et al.* 2014). Metagenomics amplicon sequencing analysis using Next Generation Sequencing (NGS) is a very useful tool to reveal the fungal community during fruit development with uncultured methods which were used in this research. This study aimed to observe the fungal community on Chokanan mango on 15-week old fruits (physiologically ripe fruits), 17-week old healthy fruits (edible ripe fruits) and 17-week old fruits with symptoms of stem end rot and anthracnose disease. To the best of our knowledge, this is the first study to investigate the variation of fungal

communities on Chokanan mango from freshly harvested to ripe fruits both for healthy and sick mangos with stem end rot and anthracnose symptoms.

Materials and Methods

Sample collection and incubation for natural infection

Chokanan mango was freshly harvested from the plantation of PT Galasari Gunung Sejahtera, Gresik, East Java, Indonesia. Physiologically ripe Chokanan mango (15-weeks old) from unbagged branches were harvested and sent to the Laboratory of Control Technology, Faculty of Agriculture Universitas Gadjah Mada, Yogyakarta, Indonesia. In the laboratory, the mangos were incubated in an air ventilated room for 2 weeks until the consumption ripe fruit phase (17-weeks old). They were observed for healthy fruits, or fruits with stem end rot and anthracnose disease symptoms.

Genomic DNA extraction and metagenomic amplicon sequencing

Fruits at 15-weeks of age (H15.ITS) were prepared for genomic DNA extraction soon after the fruits reached the laboratory. Fruits at 17-weeks of age were selected for their healthy condition (H17.ITS), stem end rot symptoms (S17.ITS) and anthracnose symptoms (A17.ITS), for genomic DNA extraction separately. The extraction was carried out from the peel of the Chokanan mango fruit using ZymoBIOMICS™ DNA Miniprep Kit (Zymoresearch, USA) according to the protocol. Six mango fruits per condition were sampled for genomic DNA extraction. Quality control (QC) for the NGS process was performed by using Nanodrop 2000 spectrophotometer (Nano-drop Technologies, Wilmington, DE, USA) with QC standard as much as 20 $\mu\text{l} \cdot \text{ng}^{-1}$ for minimum genomic deoxyribonucleic acid (DNA) concentration and 1.8 for minimum genomic DNA purity. Three QC passed samples were pooled into one tube for amplicon metagenomic sequencing. The library preparation and amplicon metagenomic sequencing were delegated to Novogene Co. Ltd. (Beijing, China), using high-throughput sequencer, Illumina Novaseq 6000. Amplified region for fungal Internal Transcribed Spacer (ITS) was conducted using primers ITS5-1737F (GGAAGTAAAAGTCGTAACAAGG) and ITS2-2043R (GCTGCGTTCTTCATCGATGC) targeting ITS 1 region with a fragment length of 200–400 bp (Ranjard *et al.* 2001; Deng *et al.* 2019; Widiastuti *et al.* 2023). Because of high variability and suitability for the shorter reads, made feasible by the paired-end Illumina, the ITS1 region was selected.

Bioinformatics analysis

Sequencing data processing

Paired-end reads were assigned to samples based on their unique barcodes and truncated by cutting off the barcode and primer sequences. Paired-end reads were merged using FLASH (V1.2.7) (Magoč and Salzberg 2011). Quality filtering on the raw tags was performed under specific filtering conditions to obtain high-quality clean tags (Bokulich *et al.* 2013) according to the Qiime (V1.7.0) (Caporaso *et al.* 2010) quality-controlled process. The tags were compared with the reference database (Unite V8.2 database using UCHIME algorithm (UCHIME Algorithm) (Edgar *et al.* 2011) to detect chimera sequences. Afterwards, the chimera sequences were removed to obtain the Effective Tags.

Operational Taxonomic Units (OTUs) cluster and taxonomic annotation

Sequence analyses were performed by UPARSE software (Edgar 2013) using all the effective tags. Sequences with $\geq 97\%$ similarity were assigned to the same OTUs. A representative sequence for each OTU was screened for further annotation. Sequences analysis was performed by blast with blastall (Version 2.2.25) and Unite V8.2 database (Kõljalg *et al.* 2013) for species annotation at each taxonomic rank (kingdom, phylum, class, order, family, genus, species). MUSCLE was used to do multiple sequence alignment to obtain the phylogenetic relationship of all OTUs representative sequences (Edgar 2004, 2021). OTUs abundance information was normalized using a standard sequence number corresponding to the sample with the least sequences. Subsequent alpha and beta diversity analyses were achieved based on this output normalized data.

Alpha and beta diversity

Alpha diversity was applied in analyzing the complexity of biodiversity for a sample through six indices, including Observed-species, Chao1, Shannon, Simpson, Abundance-based coverage estimators (ACE), and Good-coverage. All these indices in our samples were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3). Beta diversity analysis was used to evaluate the differences of samples in species complexity. Beta diversity on both weighted and unweighted UniFrac was calculated by QIIME software (Version 1.7.0). Principal Coordinate Analysis (PCoA) was performed to get principal coordinates and visualize from complex, multidimensional data. A distance matrix of weighted or unweighted UniFrac among samples obtained previously was transformed into a new set of orthogonal axes. The maximum variation factor was demonstrated by the first principal coordinate, the second maximum one by the second principal coordinate, and so on. PCoA analysis was displayed by the WGCNA package, stat packages, and ggplot2 package in R software (Version 2.15.3).

Results

Stem end rot and anthracnose disease symptoms

Chokanan healthy fruits have a green to bright yellow skin at 15 to 17-weeks (Fig. 1A and B) with a pleasant aroma. While ripening, some fruits got stem end rot disease symptoms which were characterized by a dark brown lesion starting from the stem end area which became soft rot and quickly spread to the mango surface.



Fig. 1. Chokanan mango fruit development from 15 to 17-weeks of age. A – freshly harvested healthy fruits at 15-weeks old; B – 17-week old healthy fruits; C – 17-week old fruits with stem end rot disease symptoms; D – 17-week old fruits with anthracnose disease symptoms

When the fruits were cut off, the dark brown area usually got rotten, wet, and therefore were not edible (Fig. 1C). While anthracnose disease was characterized by dry and sunken black spots, which slowly became bigger and spread into a wider area (Fig. 1D). Stem end rot symptoms developed from the stem end area while spots of anthracnose could emerge from every part of mango skin although it mainly occurred at the bottom of fruits.

Fungal communities in illumina sequencing

After quality evaluation and removing chimeric sequences, total effective tags, annotated tags and number of Operational Taxonomic Units (OTUs) of fruit conditions which were healthy 15-week old fruit (H15.ITS), healthy 17-week old fruit (H17.ITS), fruit with stem end rot symptoms at 17-weeks (S17.ITS) and fruit with anthracnose symptoms at 17-weeks (A17.ITS) were shown in Figure 2. The average of total tags from all those conditions was 131.462 tags with an average of 130.790 taxon tags, 0 unclassified tags and 671 unique tags, while the average of OTU numbers was 72.

The total number of OTUs detected in each sample varied from 55 (H15.ITS) to 92 (H17.ITS). These data were in line with the rarefaction curve (Fig. 3) which showed the varied abundance of fungal community species. H17.ITS consisted of the highest observed species number compared to the others, while S17.ITS

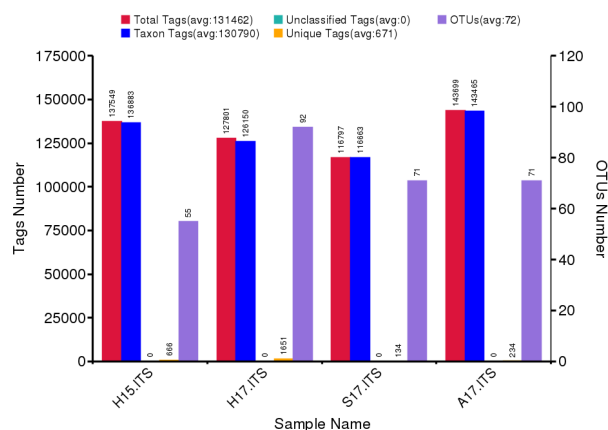


Fig. 2. Summary of the tags and OTUs number of each sample. The Y1-axis titled "Tags Number" means the number of tags. Total tags (red bars) means the number of effective tags. Taxon tags (blue bars) means the number of annotated tags. Unclassified tags (green bars) means the number of unannotated tags. Unique tags (orange bars) means the number of tags with a frequency of 1 and only occurs in one sample. The Y2-axis titled "OTUs Numbers" means the number of OTUs which displayed as "OTUs" (purple bars) in the above picture to identify the numbers of OTUs in different samples. H15.ITS: healthy 15-week old mango; H17.ITS: healthy 17-week old mango; S17.ITS: 17-week old mango with stem end rot symptoms; A17.ITS: 17-week old mango with anthracnose symptoms

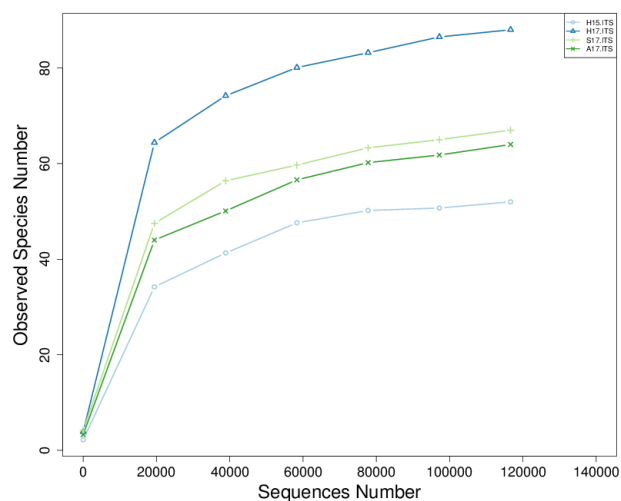


Fig. 3. Rarefaction curves of the fungal community's composition. The rarefaction curves were assembled showing the OTUs, relative to the number of total sequences. H15.ITS: healthy 15-week old mango; H17.ITS: healthy 17-week old mango; S17.ITS: stem end rot symptoms of 17-week old mango; A17.ITS: anthracnose symptoms of 17-week old mango

and A17.ITS were approximately similar to the observed species number, but H15.ITS had the lowest number. The rarefaction curve showed a flat trend in the final part indicating that each sample's sequencing data was sufficient to represent the fungal communities.

Alpha diversity and richness

OTUs generated at 97% sequence identity were regarded to be homologous in species, therefore statistical indices of alpha diversity were conducted by 97% clustering threshold. The fungal community in H17.ITS showed the highest phylogenetic diversity (PD) whole tree, ACE and Chao1 indexes with index values of 26.265; 91.722; 89.750, respectively (Table 1). These data were matched to the rarefaction curve and OTU numbers of which H17.ITS had of the highest observed species numbers.

Beta diversity analysis

Beta diversity heatmap in Figure 4 showed that H15.ITS had the highest dissimilarity coefficient of weighted UniFrac matrix compared to all samples which were H17.ITS, S17.ITS and A17.ITS, although the diversity coefficient was small not much and ranged from 0.503 to 0.517. Among 17-week samples, dissimilarity coefficient of weighted UniFrac matrix was shown by H17.ITS to S17.ITS and A17.ITS. Based on unweighted UniFrac distance matrix which showed the abundance change in rare lineages, H15.ITS had the highest dissimilarity coefficient to S17.ITS and A17.ITS, which

Table 1. Alpha diversity indices statistics of four samples on mango associated with stem end rot and anthracnose diseases

Sample name	Observed species	Shannon	Simpson	Chao1	ACE	Goods coverage	PD whole tree
H15.ITS	52	1.077	0.418	52.909	54.514	1.000	22.859
H17.ITS	88	2.128	0.637	89.750	91.722	1.000	26.265
S17.ITS	67	2.357	0.720	69.333	70.863	1.000	20.679
A17.ITS	64	1.990	0.645	65.750	68.716	1.000	21.254

H15.ITS: healthy 15-week old mango; H17.ITS: healthy 17-week old mango; S17.ITS: stem end rot symptomized 17-week old mango; A17.ITS: anthracnose symptomized 17-week old mango

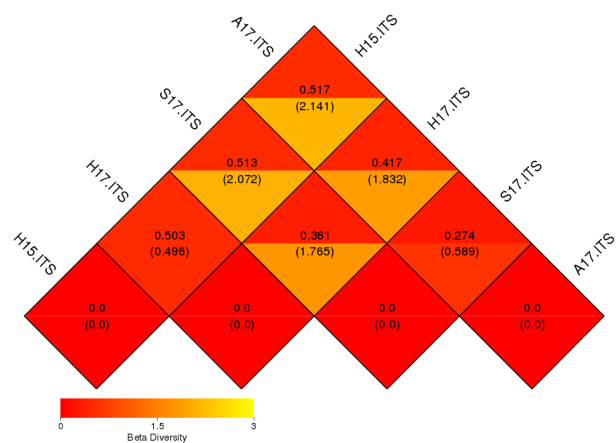


Fig. 4. Heatmap of the dissimilarity coefficient between the four samples. In the same square, the upper values are the coefficient calculated using the weighted UniFrac distance matrix, while the lower values are the unweighted UniFrac distance matrix. H15.ITS: healthy 15-week old mango; H17.ITS: healthy 17-week old mango; S17.ITS: 17-week old mango with stem end rot symptoms; A17.ITS: 17-week old mango with anthracnose symptoms

ranged from 2.072 to 2.141, while against H17.ITS, the dissimilarity coefficient was lower at 0.496.

Principal Component Analysis (PCoA) plot based on weighted UniFrac distance matrix and unweighted UniFrac distance matrix was shown in Figure 5. The PCoA results showed that, when the samples were grouped into healthy (H15.ITS and H17.ITS) and sick fruits (S17.ITS and A17.ITS), the relative abundance of fungal communities based on the population of healthy fruits was closer than the relative abundance of the sick fruits, while S17.ITS and A17.ITS was separated. When rare lineages were taken into account in the unweighted UniFrac matrix, H15.ITS had a far position from H17.ITS, while S17.ITS and A17.ITS were relatively near.

Fungal community structure and identified taxa

The Venn diagram in Figure 6 showed common and specific OTUs among samples. Each sample possessed

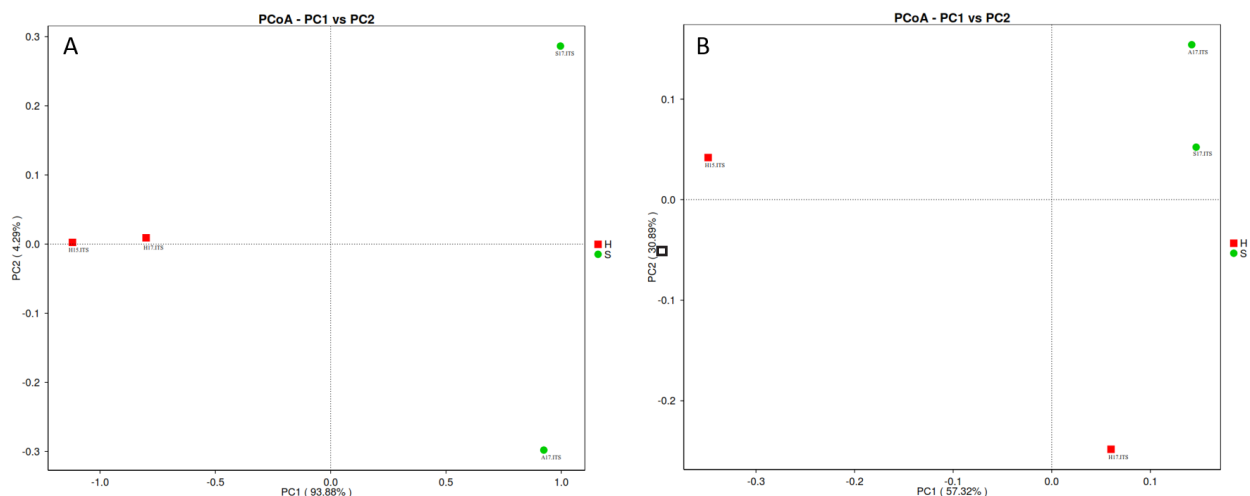


Fig. 5. A – Principal Component Analysis (PCoA) plot based on weighted UniFrac distance matrix and B – unweighted UniFrac distance matrix. H is Healthy fruits group, consisted of H15.ITS: healthy 15-week old mango; H17.ITS: healthy 17-week old mango; while S is Sick fruits group consisted of S17.ITS: 17-week old mango with stem end rot symptoms; A17.ITS: 17-week old mango with anthracnose symptoms. Red: Healthy fruits; Green: Sick fruits

23, 29, 9, and 8 specific OTUs for H15.ITS, H17.ITS, S17.ITS and A17.ITS, respectively. All samples shared 19 common OTUs which were always present in the different aged fruits and under different conditions of fruits at the same age. In the 17-week old fruits, the fungal community shared 37 common OTUs, while healthy fruits (H17.ITS) had 35 specific OTUs which possibly determined the healthy status of fruits. Both S17.ITS and A17.ITS acquired a small number of specific OTUs which were 9 (S17.ITS) and 10 (A17.ITS) which possibly define the presence of fungal pathogens which regulate fruit sickness. When the fungal communities of healthy and sick fruit samples were compared, there were 58 specific OTUs belonging to healthy fruits and 29 specific OTUs determined in the sick fruit samples. There were 60 common OTUs shared in the healthy and sick fruit samples, while 37 common OTUs were shared in the 17-week samples regardless of being healthy or sick. Those specific OTUs describing the fungal community associated with stem end rot and anthracnose disease were discussed below.

Data in Figure 7 explained that dominant taxa in the fungal community for each mango fruit condition were obviously different, both in phylum and genus of each sample, and phylum in the different fruit conditions. The Basidiomycota phylum was dominant in the healthy fruit samples both H15.ITS and H17ITS, while the Ascomycota phylum was found dominantly in sick fruit samples both S17.ITS and A17.ITS (Fig. 7A and B). Figure 7C showed that predominant fungal genera were specifically based on the condition and age of mango fruit. *Lasiodiplodia*, *Neofusicoccum*, and *Candida* were the most prevalent fungal genera linked to stem end rot disease, whereas *Colletotrichum*, *Botryosphaeria*, *Volutella*, and *Pseudofusicoccum* were the most prevalent genera linked to anthracnose disease. Those fungal genera were not found to be dominant in the healthy fruits, moreover the healthy fruits

at different ages also showed different predominant genera of the fungal community.

Discussion

Stem end rot and anthracnose disease are the two most common postharvest diseases of mangos globally and are difficult to manage. Apart from the fact that these two diseases appear during the ripening and senescence stages, both are caused by a complex of pathogens consisting of various species, which cause disease control to be difficult. Therefore, this research was conducted to determine the fungal community differences in healthy and sick mango fruits, including anthracnose and stem end rot which cause different symptoms. An early sign of mango anthracnose is sunken black spots spread over the fruit skin, which gradually accumulate in larger areas. In contrast, symptoms of stem end rot disease include soft browning which begins from the fruit stem area which become larger, covering all of the fruit skin. Although anthracnose symptoms developed as dry spots, both diseases cause rotten fruit in the late symptoms. As a mango producing country, Indonesia has a big challenge to handle these postharvest diseases to enlarge export as well as the local market.

The results showed that mango fruits in each condition, i.e., healthy 15-weeks old (H15.ITS), healthy 17-weeks old (H17.ITS), 17-week old fruit with stem end rot symptoms (S17.ITS) and 17-week old fruit with anthracnose symptoms (A17.ITS) possessed unique fungal communities which were different from each other. Healthy and mature fruits, H17.ITS contained the highest number of detected species compared to the other fruit conditions. Ripe mangos have more complex compositions of nutritional

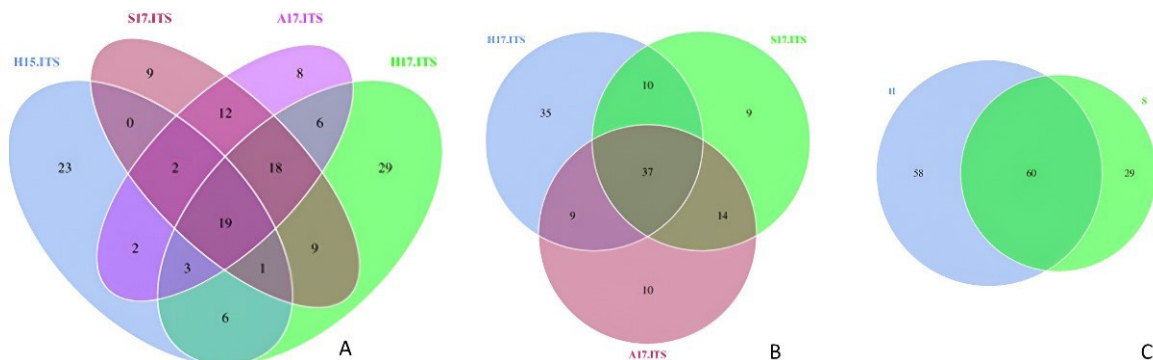


Fig. 6. Venn diagram of the fungal community among samples. Each circle represents one community sample. Values in overlapped regions signify common OTUs. The others are specific ones in each sample. H15.ITS: healthy 15-week old mango; H17.ITS: healthy 17-week old mango; S17.ITS: 17-week old mango with stem end rot symptoms; A17.ITS: 17-week old mango with anthracnose symptoms.

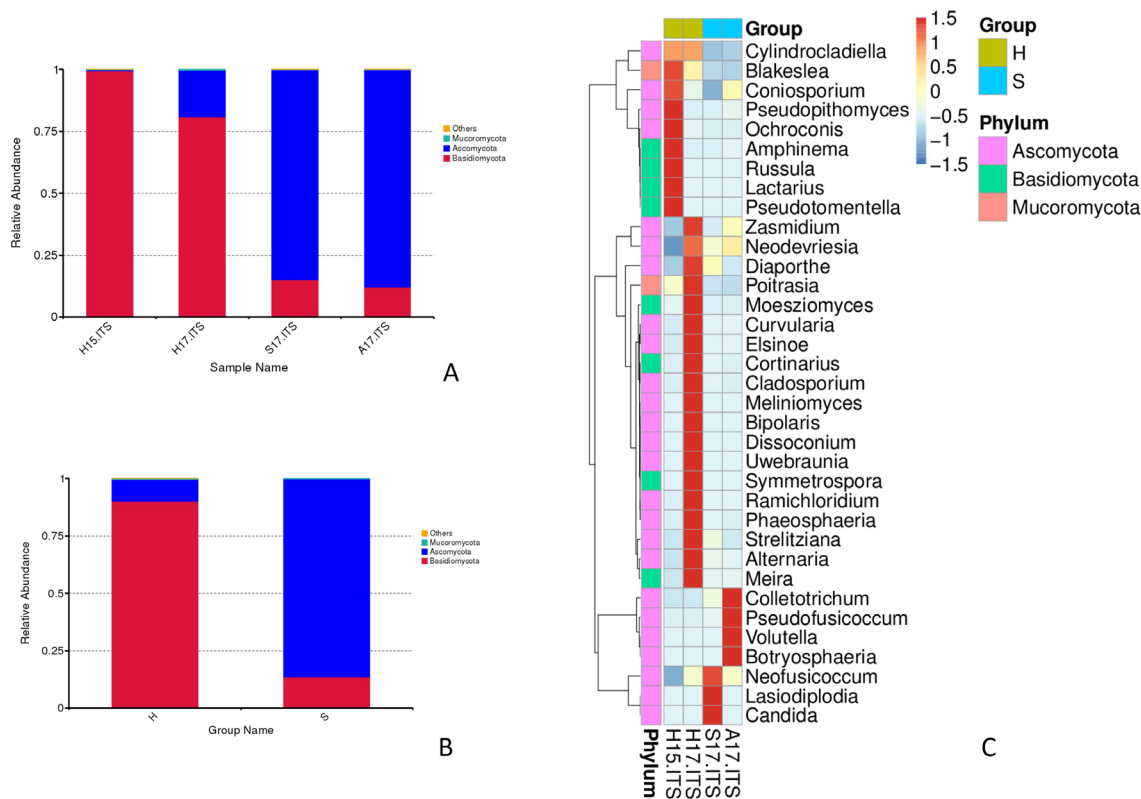


Fig. 7. A – taxonomic abundance cluster heatmap for taxa phylum abundance among samples, B – taxa phylum abundance between healthy and sick fruit samples and C – top 35 fungal genera of each sample, with sample name on the X-axis and fungal genus on the Y-axis. H is healthy fruits group, consisted of H15.ITS: healthy 15-week age mango; H17.ITS: healthy 17-week age mango; while S is Sick fruits group consisted of S17.ITS: 17-week old mango with stem end rot symptoms; A17.ITS: 17-week old mango with anthracnose symptoms.

and phytochemical components than unripe ones. Important biochemical, physiological, and structural changes take place during the growth and maturation fruit stages. These changes primarily alter the nutritional and phytochemical composition, generating softening and modifying aroma, flavor, and antioxidant capacity (Maldonado-Celis *et al.* 2019) which may influence the microbial community abundance associated with the fruit’s condition.

Based on Shannon and Simpson diversity indexes in Table 1, the composition of fungal communities of H17.ITS were not much different than S17.ITS and A17.ITS. Among samples, S17.ITS had the highest Shannon and Simpson index indicating that fungal communities in the samples were the highest in richness and evenness species compared to others. Other samples at 17-weeks of age had relatively similar Shannon and Simpson indices, which showed that during the ripening process, fungal communities of mango increased both in diversity abundance or richness and evenness. It is supported by the data of H15.ITS which showed that the fungal community of mango was low in richness as well as evenness based on Shannon and Simpson indices. Good coverage gained 100% scores for all samples,

showing that the sequencing depth was sufficient to identify the majority of the fungi present in samples. It was also confirmed by the consistency among ACE, Chao1 and observed species of each sample.

Beta diversity characterized the obvious comparison of microbial communities based on their composition to evaluate the differences between microbial communities based on weighted and unweighted UniFrac distance (Lozupone *et al.* 2011). Both data on weighted and unweighted UniFrac distance showed that H15.ITS had the highest dissimilarity coefficient compared to all samples (Fig. 4). As weighted UniFrac matrix considers relative abundance of species/taxa shared between samples, it means that the H15.ITS sample had the highest difference in species abundance information compared to other samples, continued by H17.ITS to other 17-week fruit samples. While based on unweighted UniFrac distance matrix, abundance change in rare lineages was detected on H15.ITS compared to H17. ITS, S17.ITS and A17.ITS. The dissimilarity in unique taxa was also continued by H17.ITS to S17.ITS and A17.ITS. Taken these data together it can be concluded that fruit age determines the relative abundance and specific or rare lineages of fungal communities, which continued

according to the condition of fruits at the same age and which regulate the fungal community abundance. Along with previous results, the PCoA graph (Fig. 5) showed that the relative abundance of the microbial community based on the population of healthy fruits was closer than the relative fungal abundance of sick fruits, while S17.ITS (stem end rot fruits) and A17.ITS (anthracnose fruits) were divided. In the unweighted UniFrac matrix, when the abundance or rare lineages were considered, H15.ITS was far from H17.ITS, but the sick fruits S17.ITS and A17.ITS were relatively closer, indicating that they probably shared the rare lineages present in the fungal community.

Figure 7 showed the uniqueness of taxonomic taxa phylum abundance among samples. On H17.ITS, the number of fungal communities in Ascomycota phylum increased approximately 20% compared to H15.ITS (Fig. 7A) which was shown in blue in the bar chart. On S17.ITS and A17.ITS samples, fungal communities in Ascomycota phylum occupied more than 80% which presumably contributed in the disease development of stem end rot and anthracnose. The fungal composition in H15.ITS was dominated by genera such as: *Blakeslea* (Mucoromycota), *Coniosporium*, *Pseudopithomyces*, *Ochroconis* (Ascomycota), *Amphinema*, *Russula*, *Lactarius*, *Pseudotomentella* (Basidiomycota) which mainly play roles as ubiquitous environmental fungi such as decomposers (Lee et al. 2018), and were not reported as plant pathogens. Fungal communities in H17.ITS consisted of 19 out of 35 dominant genera showed the abundance of fungal diversity which possibly support the health of ripe fruits. Based on OTUs alignment analysis, some species were reported to have a role as plant pathogens such as *Curvularia clavate* (OTU41), *Alternaria alternata* (OTU34), *Elsinoe pongamiae* (OTU21), *Diaporthe arengae* (OTU41) (Fan et al. 2017; Lim et al. 2019; Kee et al. 2020). However many other genera which played different roles were also present, to maintain the good condition of fruits. In S17.ITS and A17.ITS, the dominant fungal genera were well recognized as plant pathogens such as: *Lasiodiplodia*, *Neofusicoccum*, *Colletotrichum*, *Botryosphaeria*, *Volutella*, and *Pseudofusicoccum*. Data showed that these genera play important roles in the development of stem end rot and anthracnose disease on Chokanan mango. Based on OTUs alignment, some species found dominant in association with stem end rot disease in this study were *Lasiodiplodia theobromae* (OTU89, 132, 134, 141, 142), *Neofusicoccum cordaticola* (OTU20) and *N. mangiferae* (OTU60). Dominant species which were associated with mango anthracnose disease were *Colletotrichum gloeosporioides* (OTU2, 39, 76), *Botryosphaeria corticis* (OTU53), *Volutella* sp. (OTU65), and *Pseudofusicoccum violacearum* (OTU8). *Candida insectorum* (OTU22) was found to be associated with stem end rot disease, however

this species is common as a soil inhabitant (Kanti and Sumerta 2016). Since this alignment used sequencing provided by OTUs, further molecular identification based on fungal culture was needed to confirm if they belong to a species complex, such as *C. gloeosporioides* species complex as well as the *Botryosphaeria* family. *Lasiodiplodia theobromae* and *Neofusicoccum* spp. were frequently the main pathogens of mango stem end rot (Diskin et al. 2017; Galsurker et al. 2018; Widiastuti et al. 2023), while *Colletotrichum* spp. was widely reported as the main pathogen of mango anthracnose (Tovar-Pedraza et al. 2020). However this study found that some other species also contributed collectively in the specific disease symptoms. It was confirmed that fungal communities associated with stem end rot and anthracnose disease on mango was unique, and specific species contribute in particular disease development. However, based on laboratory observation, mixed infection also occurred, simultaneously generating more severe disease symptoms (Widiastuti et al. 2023). This study found that based on the OTUs alignment, some species were dominant to associate with stem end rot disease, such as: *L. theobromae*, *N. cordaticola* and *N. mangiferae*, while *C. gloeosporioides*, *B. corticis*, *Volutella* sp., and *P. violacearum* were predominant species distinctively associated with mango anthracnose. Some species in genus *Botryosphaeria*, *Neofusicoccum*, *Pseudofusicoccum* were reported to cause die back and canker disease in mango (Rezgui et al. 2018; Puig and Winterstein 2021) but still have not been reported in Indonesia. Since mango is an important global trading commodity, this information is significant in biosecurity and further research on comprehensive pathogen diagnosis and multigene-based molecular identification of fungal culture needs to be conducted.

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References

- Azhar M.H., Mohd Asrul S., Johari S., Tengku A.B., Malik T.M. 2013. Variation study on morphological characters among *Mangifera indica* L. 'Chokanan' for development of superior mango clone. *Acta Horticulturae* 1012: 305–313. DOI: <https://doi.org/10.17660/ActaHortic.2013.1012.38>

- Benatar G.V., Wibowo A., Suryanti. 2021. First report of *Colletotrichum asianum* associated with mango fruit anthracnose in Indonesia. *Crop Protection* 141: 105432. DOI: <https://doi.org/10.1016/j.cropro.2020.105432>
- Bokulich N.A., Subramanian S., Faith J.J., Gevers D., Gordon J.I., Knight R., Mills D.A., Caporaso J.G. 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods* 10 (1): 57–59. DOI: <https://doi.org/10.1038/nmeth.2276>
- Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Peña A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenkov T., Zaneveld J., Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7 (5): 335–336. DOI: <https://doi.org/10.1038/nmeth.f.303>
- Deng Z.S., Liu X.D., Zhang B.C., Jiao S., Qi X.Y., Sun Z.H., He X.L., Liu Y.Z., Li J., Chen K.K., Lin Z.X., Jiang Y.Y. 2019. The root endophytic fungi community structure of *Pennisetum sinense* from four representative provinces in China. *Microorganisms* 7 (9): 332. DOI: <https://doi.org/10.3390/microorganisms7090332>
- Diskin S., Oleg F., Dalia M., Samir D., Dov P., Noam A. 2017. Microbiome alterations are correlated with occurrence of postharvest stem-end rot in mango Fruit. *Pythobiome Journal* 1: 117–127. DOI: <https://doi.org/10.1094/PBIOMES-05-17-0022-R>
- Edgar R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32 (5): 1792–1797. DOI: <https://doi.org/10.1093/nar/gkh340>
- Edgar R.C., Hass B.J., Clemente J.C., Quince C., Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27 (16): 2194–2200. DOI: <https://doi.org/10.1093/bioinformatics/btr381>
- Edgar R.C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* 10 (10): 996–998. DOI: <https://doi.org/10.1038/nmeth.2604>
- Edgar R.C. 2021. MUSCLE V5 Enables improved estimates of phylogenetic tree confidence by ensemble bootstrapping. *bioRxiv*. DOI: <https://doi.org/10.1101/2021.06.20.449169>
- Fan X.L., Barreto R.W., Groenewald J.Z., Bezerra J.D.P., Pereira O.L., Cheewangkoon R., Mostert L., Tian C.M., Crous P.W. 2017. Phylogeny and taxonomy of the scab and spot anthracnose fungus *Elsinoë* (Myriangiiales, Dothideomycetes). *Studies in Mycology* 87: 1–41. DOI: <https://doi.org/10.1016/j.simyco.2017.02.001>
- Galsurker O., Sonia D., Dalia M., Oleg F., Noam A. 2018. Fruit stem-end rot. *Horticulturae* 4 (4): 50. DOI: <https://doi.org/10.3390/horticulturae4040050>
- Galsurker O., Diskin S., Duanis-Assaf D., Doron-Faigenboim A., Maurer D., Feygenberg O., Alkan N. 2020. Harvesting mango fruit with a short stem-end altered endophytic microbiome and reduce stem-end rot. *Microorganisms* 8: 558. DOI: <https://doi.org/10.3390/microorganisms8040558>
- Kanti A., Sumerta I.N. 2016. Diversity of xylose assimilating yeast from the island of Enggano, Sumatera, Indonesia. *Berita Biologi* 15 (3): 207–215. DOI: <https://doi.org/10.14203/beritabiologi.v15i3.2215>
- Kee Y.J., Zakaria L., Mohd M.H. 2020. *Curvularia asianensis* and *Curvularia eragrostidis* associated with leaf spot of *Sansevieria trifasciata* in Malaysia. *Journal of Phytopathology* 168 (5): 290–296. DOI: <https://doi.org/10.1111/jph.12890>
- Kiloes A.M., Muflikh Y.N., Joyce D., Ammar A.A. 2023. Understanding the complexity of the Indonesian fresh mango industry in delivering quality to markets: A systems thinking approach. *Food Policy* 118: 102497. DOI: <https://doi.org/10.1016/j.foodpol.2023.102497>
- Köljalg U., Nilsson R.H., Abarenkov K., Tedersoo L., Taylor A.F., Bahram M., Bates S.T., Bruns T.D., Bengtsson-Palme J., Callaghan T.M., Douglas B., Drenkhan T., Eberhardt U., Dueñas M., Grebenc T., Griffith G.W., Hartmann M., Kirk P.M., Kohout P., Larsson E., Lindahl B.D., Lücking R., Martín M.P., Matheny P.B., Nguyen N.H., Niskanen T., Oja J., Peay K.G., Peintner U., Peterson M., Pöldmaa K., Saag L., Saar I., Schüßler A., Scott J.A., Senés C., Smith M.E., Suija A., Taylor D.L., Telleria M.T., Weiss M., Larsson K.H. 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 22 (21): 5271–5277. DOI: <https://doi.org/10.1111/mec.12481>
- Li Q., Bu J., Shu J., Yu Z., Tang L., Huang S., Guo T., Mo J., Luo S., Solangi G.S., Hsiang T. 2019. *Colletotrichum* species associated with mango in southern China. *Scientific Reports* 9 (1): 18891. DOI: <https://doi.org/10.1038/s41598-019-54809-4>
- Lim L., Mohd M.H., Zakaria L. 2019. Identification and pathogenicity of *Diaporthe* species associated with stem-end rot of mango (*Mangifera indica* L.). *European Journal of Plant Pathology* 155: 687–696. DOI: <https://doi.org/10.1007/s10658-019-01800-4>
- Lee H., Park J.Y., Wisitrassameewong K., Kim M.J., Park M.S., Kim N.K., Lee J.K., Lim Y.W. 2018. First report of eight milkcap species belonging to *Lactarius* and *Lactifluus* in Korea. *Mycobiology*. 46 (1): 1–12. DOI: <https://doi.org/10.1080/12298093.2018.1454012>
- Lozupone C., Lladser M.E., Knights D., Stombaugh J., Knight R. 2011. UniFrac: an effective distance metric for microbial community comparison. *The ISME Journal* 5: 169–172. DOI: <https://doi.org/10.1038/ismej.2010.133>
- Lu M., Yang Z., Ma L., Li Q., Huang S., Tang L., Guo T., Mo J., Hsiang T. 2022. First report of postharvest anthracnose of mango fruit caused by *Colletotrichum plurivorum* in China. *Journal of Plant Pathology* 104: 839–840. DOI: <https://doi.org/10.1007/s42161-022-01047-9>
- Maldonado-Celis M.E., Yahia E.M., Bedoya R., Landázuri P., Loango N., Aguillón J., Restrepo B., Guerrero Ospina J.C. 2019. Chemical composition of Mango (*Mangifera indica* L.) fruit: nutritional and phytochemical compounds. *Frontiers in Plant Science* 10: 1073. DOI: <https://doi.org/10.3389/fpls.2019.01073>
- Magoč T., Salzberg S.L. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27 (21): 2957–2963. DOI: <https://doi.org/10.1093/bioinformatics/btr507>
- Puspitasari D. 2023. Promising Indonesian mango export opportunities. Ministry of Agriculture, Republic Indonesia. [Available on: <https://hortikultura.pertanian.go.id/peluang-ekspor-mangga-indonesia-menjanjikan/>] [Accessed: 28 October 2023]
- Ranjard L., Poly F., Lata J.C., Mougél C., Thioulouse J., Nazaret S. 2001. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. *Applied and Environmental Microbiology* 67 (10): 4479–4487. DOI: <https://doi.org/10.1128/AEM.67.10.4479-4487.2001>
- Rezgui A., Vallance J., Ghnaya-Chakroun A.B., Bruéz E., Dridi M., Demasse R.D., Rey P., Sadfi-Zouaoui N. 2018. Study of *Lasiodiplodia pseudotheobromae*, *Neofusicoccum parvum* and *Schizophyllum commune*, three pathogenic fungi associated with the grapevine trunk diseases in the north of Tunisia. *European Journal of Plant Pathology* 152: 127–142. DOI: <https://doi.org/10.1007/s10658-018-1458-z>
- Pinto C., Pinho D., Sousa S., Pinheiro M., Egas C., Gomes A.C. 2014. Unravelling the diversity of grapevine microbiome. *PLoS ONE* 9 (1): e85622. DOI: <https://doi.org/10.1371/journal.pone.0085622>
- Puig A.S., Winterstein M.C. 2021 *Neofusicoccum batangarum* causing dieback of mango (*Mangifera indica*) in Florida. *Agriculture* 11: 853. DOI: <https://doi.org/10.3390/agriculture11090853>
- Statistics Indonesia. 2023. Production of Fruits 2022. [Available on: <https://www.bps.go.id/subject/55/hortikultura.html#subjekViewTab3>] [Accessed: 10 August 2023]

- Taylor M.W., Tsai P., Anfang N., Ross H.A., Goddard M.R. 2014. Pyrosequencing reveals regional differences in fruit-associated fungal communities. *Environmental Microbiology* 16: 2848–2858. DOI: <https://doi.org/10.1111/1462-2920.12456>
- Tovar-Pedraza J.M., Mora-Aguilera J.A., Nava-Díaz C., Lima N.B., Michereff S.J., Sandoval-Islas J.S., Câmara M.P.S., Téliz-Ortiz D., Leyva-Mir S.G. 2020. Distribution and pathogenicity of *Colletotrichum* species associated with mango anthracnose in Mexico. *Plant Disease* 104: 137–146. DOI: <https://doi.org/10.1094/PDIS-01-19-0178-RE>
- Uddin, M.N., Shefat, S.H.T., Afroz, M., Moon, N.J. 2018. Management of anthracnose disease of mango caused by *Colletotrichum gloeosporioides*: a review. *Acta Scientific Agriculture* 2: 169–177.
- Widiastuti A., Suryanti, Giovanni A.C., Santika I.A., Paramita N.R. 2023. Fungal community associated with mixed infection of anthracnose and stem end rot diseases in Chokanan Mango. *Biodiversitas* 24: 2163–2170. DOI: <https://doi.org/10.13057/biodiv/d240428>