ARTICLE

Detection of *Alternaria alternata* in post-harvest onion and grapes and the assessment of their phylogenetic relationships and mating potential

Jonas P. Biscocho[1,2], Monica Pauline R. Baysa[1], & Leilani S. Dacones*[1,2]

[1]Institute of Biology, University of the Philippines Diliman, Quezon City, Philippines
[2]Natural Sciences Research Institute, University of the Philippines Diliman, Quezon City, Philippines

ABSTRACT

Postharvest diseases account for economic losses due to damages to crops that become unmarketable or decrease in quality. *Alternaria alternata* is an asexual fungus and a postharvest pathogen of various crops. Here, we described the detection of *A. alternata* isolates from grapes and onion in the Northern Philippines. A total of eleven (11) isolates were characterized for morphological attributes, estimated the genetic diversity, and consequently assessed for their mating potential. In addition, 29 whole genome assemblies of *A. alternata*, *A. tenuissima*, and *A. arborescens* were included in the analyses for sequence comparison. Cultural and morphological characterization revealed variations among the *A. alternata* isolates, with no distinct patterns associated with the host of origin or geographic location. Phylogenetic inferences showed a monophyletic clade for all *A. alternata* with ITS and *AA*-specific loci. However, the haplotype network based on parsimony exhibited distinct haplotypes and loops in the network indicative of reticulate relationships. Furthermore, isolates from the Northern Philippines all manifested only one mating type idiomorph signifying *A. alternata* as a heterothallic fungus. These results further provide inferences on the potential diversity of *A. alternata* isolates in the Philippines that might confer phenotypic advantages such as management strategies implemented against postharvest fungal pathogens.

*Corresponding author
Email Address: lsdacones@up.edu.ph
Date received: July 20, 2022
Date revised: October 09, 2022
Date accepted: October 21, 2022

INTRODUCTION

Postharvest loss is described as the decreased quality and quantity of economically important crops, and this is affecting agricultural crop production worldwide. In the United States alone, various research and surveys from different organizations estimated that the postharvest losses of fruits and vegetables range from 40-50% (Alkan & Fortes, 2015). Plant pathogens responsible for postharvest loss are composed of bacteria, e.g., *Erwinia* and *Pseudomonas*, and then fungi *Alternaria, Aspergillus, Botrytis, Colletotrichum, Diplodia, Monilinia, Penicillium, Phomopsis, Rhizopus, Mucor,* and *Sclerotinia* (Singh & Sharma, 2018). The occurrence of infection is influenced by factors such as pathogenicity, host response, and the environment (Alkan & Fortes, 2015). These factors can be further classified into two: internal factors (i.e., harvesting, storage,

KEYWORDS

Alternaria alternata, mating type idiomorphs, onion leaf blight, fruit/bunch rot of grape, phylogenetic diversity

transportation, biological, and microbiological) and external factors (i.e., environmental, and socio-economic) (Kiaya, 2014). Postharvest pathogens commonly cause crop production problems in the Philippines, particularly storage. However, there is limited information on the different fungi identified in different crops and produce in the country and their corresponding biology and distribution. According to the Bureau of Agriculture and Fisheries Standards (BAFS), the Philippines possesses a total grape area of 389 hectares. In 2019, it produced 214 tons, but the country is still importing from other countries despite these values. On the other hand, onion production from 2016-2020, there was an observed increase in production by 19% (Philippine Statistics Authority). However, the production of onion and grapes in the Philippines is negatively impacted by various diseases, including postharvest diseases caused by fungal plant pathogens.

The Philippines, being a tropical country, is prone to different fungal plant pathogens as it exhibits an optimal ecological parameter where they can thrive and cause damage if neglected. Some of the few examples of fungal pathogens reported causing postharvest disease in the Philippines are *Colletotrichum scovillei*, which was determined to cause anthracnose in chili farms in Batangas (Cueva *et al.*, 2018). *Fusarium fujikoroi* from rice, *Aspergillus sp.,* and *Penicillium sp.* are common fungal pathogens in Philippine coffee (Cruz *et al.,* 2013; Alvindia & De Guzman, 2016; Balendres *et al.*, 2019). Onion, one of the highest-value crops in the Philippines, is susceptible to various fungal pathogens such as *Rhizoctonia solani, Fusarium* spp., *Sclerotium rolfsii, Aspergillus* sp., and *Penicillium* sp. (Santiago *et al.,* 2018). These pathogens are the primary postharvest pathogens that cause detrimental damage to onion crops resulting to decreasing quality and crop yield. However, in grapes, there is still limited information on grape diseases caused by postharvest fungal pathogens reported in the country. Most fungal pathogens thrive in either tropical or subtropical regions like our country (Singh & Sharma, 2018). Proper identification and understanding of the pathogen diversity in a given local area are critical in assessing the predominant populations that, in turn, would help develop appropriate control methods.

Alternaria alternata is a fungal pathogen that causes postharvest diseases such as fruit rot and leaf blight on grapes and onions. In grapes, practical manifestation in those infected with *A. alternata* is the presence of a dark gray to black mycelium on the surface that may spread. At the same time, its symptoms depict a discoloration of an entire bunch (Ghuffar *et al.,* 2018). While in onion, it manifests as a dark circular spot with a distinct concentric ring surrounding it (Fernández J *et al.,* 2011). Typical colonies of *A. alternata* manifest an olive-green color and the presence of concentric rings at the back when inoculated in potato dextrose agar (PDA). Sporulation is primarily characterized by forming conidial chains that range from 6-14 conidia in length, which can further develop secondary or tertiary chains. Single conidia (20-50um long) are usually ovate and are divided by transverse and vertical walls. Hyphae and conidiophores are light brown and septate (Troncoso-Rojas & Tiznado-Hernandez, 2014).

As current research is now gearing up to a molecular approach, molecular identification of *A. alternata* is also being conducted in various countries such as by constructing a phylogenetic tree based on a multilocus approach. A study was conducted by using different conserve loci that is commonly used to identify *Alternaria* species. The concatenated phylogenetic tree was able to divide into two major clusters (*A. arborescens* and *A. alternata* clusters). Focusing on *A. alternata* clade, the said fungal species is genetically diverse as no specific grouping of isolates were observed that correlate with plant host or geographical location even using multilocus approach and there are some isolates that are left unidentified despite using multilocus approach (Matic *et al.,* 2020). *A. alternata* is classified under Phylum *Ascomycota,* Class *Dothideomycetes*, and Family *Pleosporaceae*. The same group with the known asexual fungus causing postharvest diseases such as *Botrytis* sp., *Penicillium* sp., *Alternaria* sp., *Colletotrichum* s p., *Fusarium* sp., and *Aspergillus* sp. are causing a similar impact on crops as a postharvest pathogen (Chen *et al.,* 2022). *A. alternata* has previously been characterized to reproduce asexually strictly. However, recent studies suggest that *A. alternata* may undergo sexual reproduction due to mating type idiomorphs (*MAT1-1* and *MAT1-2*). In China, the researchers determined that *A. alternata* isolated from potato production areas exhibited clonal isolates, high genotypic diversity, and an equal frequency of the mating type idiomorphs. These were also observed in *A. alternata* isolates from citrus in Florida, which indicates that recombination or sexual reproduction is a factor in evolutionary changes in this fungal species (Meng *et al.,* 2015; Stewart *et al.,* 2013).

This study focused on the detection of *A. alternata* in onion and grapes from the Philippines, the subsequent morphological and molecular characterization of isolates, and determining their mating potential. This information is essential in assessing the genetic diversity and consequently would help in understanding the roles of fungal pathogens in the epidemiology of postharvest diseases, or for instance, how populations of *A. alternata* in onion and grapes are related to each other. Moreover, knowledge of the pathogen biology and diversity would provide insights into the mode of dispersal and inoculum sources. Detection of the mating type idiomorphs present in *A. alternata* onion and grapes from the Philippines would provide inferences on the possible involvement of sexual reproduction in genetic diversity and novel genotypes for virulence and pathogenicity.

MATERIALS AND METHODS

Sample collection and isolation of *Alternaria alternata*

Due to the travel restrictions imposed to minimize COVID-19 transmission, diseased samples were obtained directly from local markets and shipped from storage facilities of local growers. The symptomatic plant tissues were subjected to surface sterilization prior to cultivation in quarter-strength potato dextrose agar (qPDA) (Sumabat *et al.,* 2018). Briefly, each symptomatic tissue (leaf or fruit) was washed in running water, then cut into four pieces prior to washing in 0.6% sodium hypochlorite for one minute and repeatedly washed in sterile distilled water (SdH$_2$O) for one minute. Next, the four cutout samples were placed equidistantly in a qPDA plate and stored in the dark at room temperature for 3-5 days. For purification of *A. alternata*, an initial ocular inspection was performed to identify colony characteristics that resemble *A. alternata*, which were then transferred to a new plate and stored at room temperature for seven days. After seven days, fungal cultures were evaluated using different parameters to help in the preliminary ID using colony morphology, such as the presence of brownish color colony and concentric rings.

Cultural and morphological characterization of *A. alternata* isolates

Purified cultures were further examined, subcultured in new plated qPDA, and stored in the dark at room temperature for seven days. Then, each isolate was prepared for observation under the microscope to characterize fungal structures, including conidia, conidiophore, and hyphae. Characteristics measured and described include shape, color, and size. In addition, colony morphology was characterized by determining their shape, presence of concentric rings, color, texture, growth, and rate. A 5mm diameter plug was retrieved from an initial culture and then inoculated in qPDA in the dark at room temperature for seven days in duplicates to determine the growth and the rate. Colony

growth was measured using a digital caliper on days 3,5 and 7 marks, and the average size of each isolate was used.

DNA extraction, PCR amplification, sequencing

Representative *A. alternata* isolates identified based on morphological characteristics from the previous method were processed for DNA extraction using E.Z.N.A. Fungal DNA Mini Kit following the manufacturer's protocol. Briefly, 100mg of 7-day-old *A. alternata* was used as fungal material. Then, the sample was added with 600ul lysis buffer (FG1 buffer) in 1.5ml microcentrifuge tubes and vortexed. Next, the sample was incubated in a heat block at 65°C for ten minutes and added 140ul glacial acetic acid (FG2 buffer) after incubation. After five minutes, the sample was centrifuged at 10,000 x g for 10 minutes. The supernatant was transferred to a new 1.5ml microcentrifuge tube and added 0.7 volume of isopropanol to precipitate the DNA and was centrifuged at 10,000 x g for two minutes. The supernatant was separated from the produced DNA pellet and was air dried for one minute. Three hundred microliters of sterile deionized water heated to 65°C were added to the DNA pellet to dissolve and vortex. Four microliters of RNase were added and vortexed to remove residual RNAs in the sample. 100% ethanol and guanidine hydrochloride (FG3 buffer) was added and vortexed to mix. The sample was then put in the HiBind DNA Mini Column and collection tube and centrifuged at 10,000 x g for one minute. The produced pellet was discarded, the mini-column was inserted in a new collection tube, and added 750ul of DNA buffer was centrifuged at 10,000 x g for one minute. The produced filtrate was discarded, and the same process was repeated. After that, discard the collection tube and centrifuge the empty mini-column at maximum speed for two minutes. The resulting product was then transferred to a 1.5ml microcentrifuge tube and added 100ul elution buffer, which was heated to 65°C and let sit for 3-5 minutes. DNA was stored at -20°C for long-term use.

Each DNA were then used as template to amplify two loci including the internal transcribed spacer (ITS) (ITS1 - 5'-TCCGTAGGTGAACCTGCGG-3' & ITS4 - 5'-TCCTCCGCTTATTGATATGC-3'), AA-specific loci (AAF2 - 5´- TGCAATCAGCGTCAGTAACAAAT-3' & AAR3 - 5´-ATGGATGCTAGACCTTTGCTGAT-3') (Konstantinova *et al*., 2002). For the PCR amplification, Promega GoTaq Master Mix, a ready-to-use PCR master mix which is composed of reaction buffer with pH 8.5, 400uM dATP, 400uM dGTP, 400uM dCTP, 400uM dTTP, 3mM MgCl2, and filled with nuclease-free water up to 25uL was utilized. In this master mix, 250ng concentration of DNA template and 1.0µM primer concentrations were used to make it to 25µl. Different PCR conditions were used for each locus. For ITS, Stage 1 (initial denaturation) was set to 95°C for 2mins in one cycle, stage 2 (final denaturation, annealing, and extension) was set at 95°C for 30 seconds, 55°C for 45 seconds, and 72°C for 1 minute in 35 cycles, respectively. Finally, stage 3 (final extension) was set at 72°C for 5 minutes in 1 cycle. For AA-specific, the same PCR conditions were used except for the annealing part of stage 2, which was set at 50°C for 45 seconds in 35 cycles. The PCR-amplified gene regions of both directions from each representative isolate were sent off for Sanger sequencing.

Phylogenetic Analysis

To determine the evolutionary relationships of *A. alternata* isolates across host species and geographic regions, sequences of each of the two loci of the *A. alternata* isolates are aligned and visually edited in Geneious Prime and aligned via Geneious alignment. In addition to sequences that were obtained in this study, sequences of the three nuclear loci from isolates representative of *A. alternata*, *A. tenuissima*, and *A. arborescens* were mined from GenBank for comparison. Before tree construction, a saturation or Xia test was conducted in DAMBE and determined if loci alignments could be used for phylogenetic analysis. Model testing was simultaneously performed using MEGA X (Tamura *et al*., 2021) to determine the most suitable DNA substitution model for the created sequence alignment. When these were accomplished, phylogenetic relationships were inferred by constructing phylogenetic trees using maximum likelihood (ML) in MEGA X version 10.2.6 (Tamura *et al.,*2021) and Bayesian Inference (BI) in MrBayes (Ronquist & Huelsenbeck, 2001). For the outgroups, *A. radicana* was used in AA phylogenetic tree construction based on the study of Konstantinova et al. (2001), where *Alternaria* spp. specific primers were identified and developed. Whereas for the ITS tree construction, *A. solani* was used as an outgroup as commonly being used in previous *Alternaria* phylogenetic studies and its closest but easily delineated with *A. alternata*.

Genetic Diversity of *A. alternata* Populations

Nucleotide and haplotype diversity within and among the different populations were estimated in each sequence alignment representative of each locus used for comparison. Genetic and haplotype diversity within the *A. alternata* representative species were also calculated in each locus. Haplotype (*h*), haplotype diversity (*hD*), number of segregating sites (*S*) and parsimonious sites (*P*), Watterson's Q (*Qw*), and nucleotide diversity (p) were computed using DnaSP v6.12.03 (Rozas, 2017). For visualization of evolutionary relationships of *A. alternata*, haplotype networks were constructed by creating a parsimony network in the TCS v 1.23 program (Murias dos Santos *et al*., 2016).

Detection of the mating-type idiomorph in *Alternaria alternata*

The same representative *A. alternata* isolates used from previous analyses were assayed to determine the mating type idiomorph present in each of them. PCR amplification was implemented Following the procedure described above with a difference in the PCR primers used. PCR primers include AA-MAT2-1691/AsM1-8 for *MAT1-2* (Forward – 5' - CAGCACCCCGACTACAAGTAT - 3' & Reverse – 5'-GGTCGTGAGTCGTGATCG - 3') and then ALMAT-L/AA-MAT1-867 for *MAT1-1* (Forward – 5' - GCAAGATTCTAGGCCCAACG - 3' & Reverse – 5'-TGCGGTGGGGAGTAGTGT - 3') (Stewart *et al.,* 2013). PCR amplification conditions include an initial denaturation set to 95°C at 5 minutes in one cycle, denaturation, annealing, and extension was set at 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds in 35 cycles, respectively, and then the final extension was set at 72°C for 10 minutes in 1 cycle. The PCR-amplified gene regions of both directions from each representative isolate were sent off for Sanger sequencing.

Maximum likelihood and Bayesian inference phylogenetic trees were also constructed using MEGA X version 10.2.6 (Tamura *et al.,*2021) and MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001). Genetic and haplotype diversity was measured by computing the following using DnaSP v6.12.03 (Rozas, 2017): haplotype (*h*), haplotype diversity (*hD*), number of segregating sites (*S*), and parsimonious sites (*P*), Watterson's Q (*Qw*), and nucleotide diversity (p) to determine the evolutionary relationships of *A. alternata* mating types 1-2. The evolutionary relationships of *A. alternata* were further visualized by constructing a haplotype network with a parsimony network in the TCS v 1.23 program (Murias dos Santos *et al*., 2016).

RESULTS

Cultural and morphological characteristics of *Alternaria alternata* isolates from Philippines

A total of eleven (11) *Alternaria alternata* isolates were identified using cultural and morphological characteristics. Eight (8) came from grapes sampled from a local market in Baguio City. Two were from local lab collections at the Department of Plant Pathology of the University of the Philippines Los Baños, and one was from an onion farm in Nueva Ecija (Table 1). In figure 2, colony morphology and fungal structures observed under the microscope of isolated *A. alternata* shows variation among these isolates. The general characteristic of all isolates is that they formed a round colony that shows the presence of concentric rings. However, colony morphology revealed differences in shape, color, and growth rates. For instance, in terms of shape, most of the isolates exhibited a circular shape; however, isolate AG9S1 showed an extension making it more oval or irregular. The isolates express different colony colors. Colonies of Alt1 and Alt2 showed a powdery white color surface and dark to greenish color at front and back, respectively.

Isolate Name	Host	Location
Alt1	Unidentified	University of the Philippines Los Baños
Alt2	Unidentified	University of the Philippines Los Baños
AG3	Grapes	Local Market (Baguio City)
AG5	Grapes	Local Market (Baguio City)
AG9S1	Grapes	Local Market (Baguio City)
AG10S1	Grapes	Local Market (Baguio City)
AG11S1	Grapes	Local Market (Baguio City)
AG12S1	Grapes	Local Market (Baguio City)
AG13S1	Grapes	Local Market (Baguio City)
AG14S1	Grapes	Local Market (Baguio City)
AON5S1	Onion	Nueva Ecija

Table 1: List of *Alternaria alternata* isolates collected from different hosts in the Northern Philippines

Interestingly, isolates from grapes exhibited two unique colors. AG3, AG9S1, AG10S1, and AG11S1 exhibited a greenish front and a dark center with grayish surroundings at the back, while AG5, AG12S1, AG13S1, and AG14S1 showed a dark center that is encircled by a white color mycelium in both front and back. Finally, AON5S1 expressed a powdery white front and a dark center to brownish at its back. The growth of each isolate was determined in replicates. All isolated indicated a positive growth rate.

The growth rate for each measurement on days 3, 5 and 7 ranges from 5.8mm-8.03mm, 0.36mm-0.49mm, and 0.21mm-0.39mm (Supplementary Table 1). During the 7-day incubation in qPDA, the colonies had grown at an average of 67.69mm having Alt1 and Alt2 being the highest (79.85mm) (Figure 1). Looking into the microscopic structure of each isolate, after three days of incubation, asexual structures, including hyphae, conidia, and conidiophore, are observed. Hyphae in all isolates are transparent and septate. The conidia in all isolates are observed to be ovate in shape, brownish, and divided by transverse and vertical walls. Isolates AG3, AG9S1, AG10S1, and AG11S1 were observed to form conidial chains consisting of 4-6 conidia interconnected by conidiophores, while others did not manifest this characteristic.

Sequences of *Alternaria alternata* isolates and other *Alternaria* sp. from NCBI

In addition to the 11 *A. alternata* isolates obtained from this study, 29 whole genome assemblies from NCBI consist of three different *Alternaria species: A. alternata* (16)*, A. tenuissima* (7), and *A. arborescens* (6) were included in the phylogenetic analyses. Based on the information in NCBI, each genome assembly was isolated from various hosts and locations, mostly assembled using SPAdes (58.7%) and sequenced primarily by Illumina (75.9%). The internal transcribed spacer (ITS), and *Alternaria alternata*-specific (AA) genes, were mined from these genome assemblies (Supplemental Table 2).

Phylogenetic analyses

To determine the relationship between *A. alternata* isolated from Northern Philippines and those mined from NCBI, isolates were compared based on the ITS and AA-specific gene regions. Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian Inference (BI) for each locus. Each phylogenetic tree constructed based on the ITS and AA-specific loci produced well-supported bootstrap values and posterior probabilities. In the AA-specific tree, all isolates collected from Northern Philippines and the AA-specific sequences extracted from the whole genome sequences from NCBI were observed to be clustered in one clade (Figure 3), indicating high similarity among isolates, and confirming the taxonomic classification of these isolates. The three species of *Alternaria* were included for the comparison in the phylogenetic trees based on the ITS. Results show that the tree topology of the three representatives *Alternaria* sp. sequences (*A. alternata, A. tenuissima, and A. arborescens*) had formed a monophyletic clade, and only one species of *A. arborescens* (*A. arborescens* MG786771.1) formed a different branch (Figure 4). This tree topology exhibits a discrepancy in identifying *A. alternata* among the two other species.

Genetic and Haplotype diversity

The genetic and haplotype diversity were computed to estimate variations between the *A. alternata* isolates. The number of haplotypes indicated a higher value in ITS (h = 8) than in AA-specific (h = 1). These results are also reflected in the haplotype diversity (hD = 0.127; hD = 0.071), number of segregating sites (S = 62; S = 1), number of parsimonious sites (P = 62; P = 1), Watterson's Q (Qw= 11.530; Qw= 0.257), and nucleotide diversity (p = 0.00479; p = 0.00112). Parsimony networks were constructed for the two loci (ITS and AA-specific) for determining the evolutionary relationships within *A. alternata* populations (Figure 7 and 8). It is noticeable that the network showed signs of interconnection among samples however this is contradicting to the result value of haplotype ($h=1$).

The haplotype networks based on parsimony showed that with the AA-specific locus, isolates formed distinct haplotypes but not based on the host of origin. For instance, isolates from grapes were distributed into four unique haplotypes. It can also be noted that some of the isolates from grapes grouped with those retrieved from NCBI that also were collected from grapes (Figure 7).

With the haplotype network based on ITS, isolates from Northern Philippines are grouped into two distinct haplotypes, where representative isolates from grapes formed a unique haplotype distinct with those from onion (Figure 8).

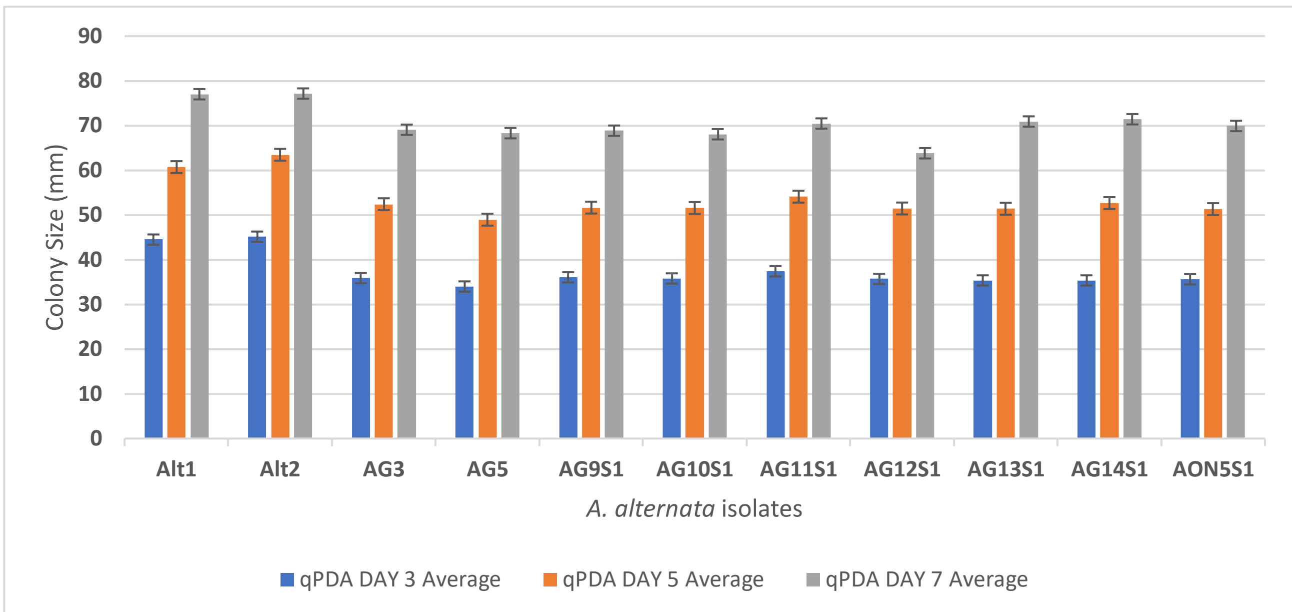
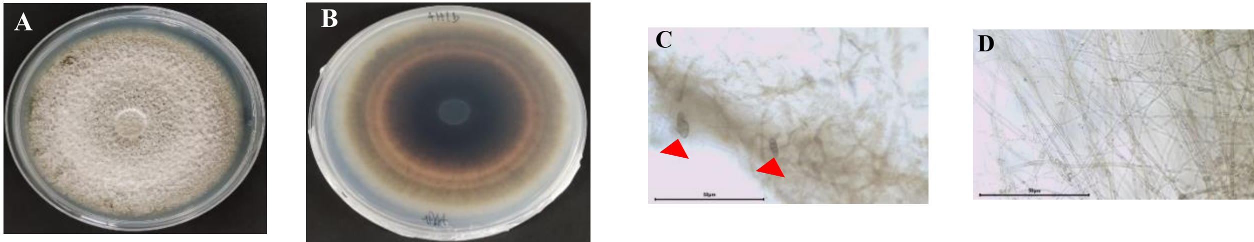
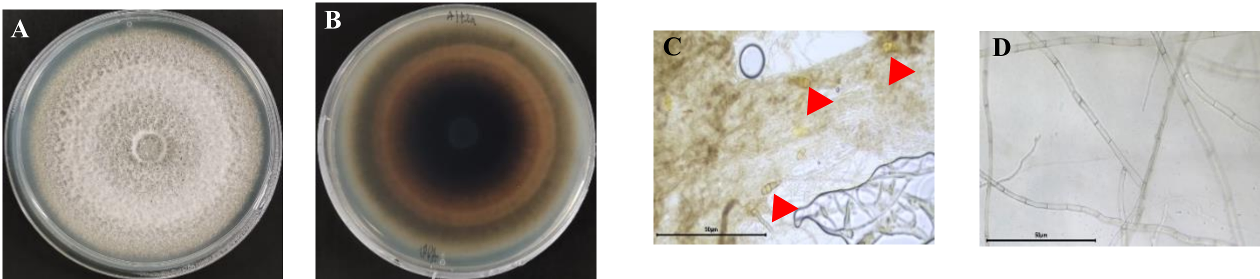


Figure 1: Colony diameter of *A. alternata* isolates in Northern Philippines. The isolates were grown in replicates of two in qPDA and incubated in the dark at room temperature for 7 days. Measurements were taken using a digital caliper at 3,5, and 7-day mark and depicted a positive growth trend.

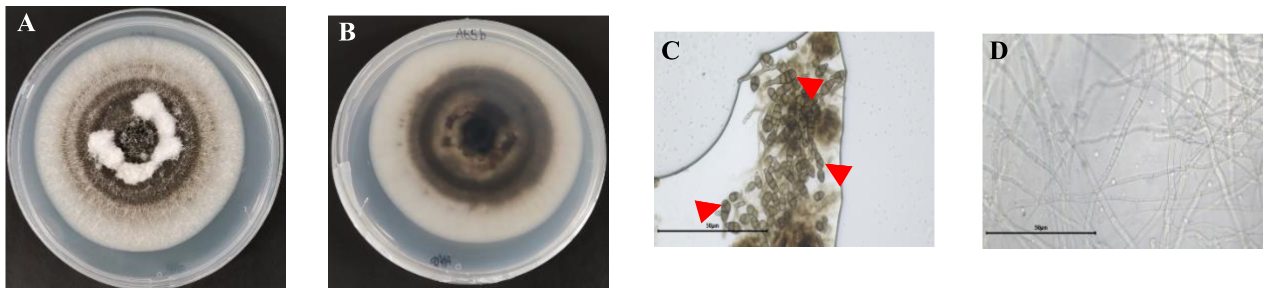
Alt1



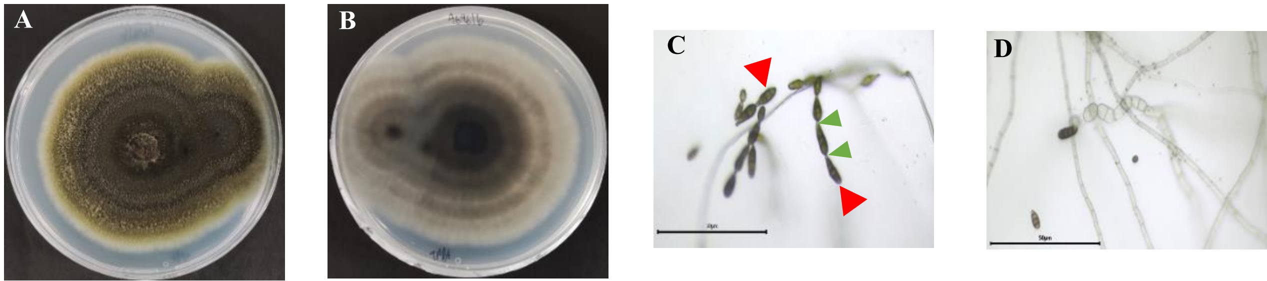
Alt2



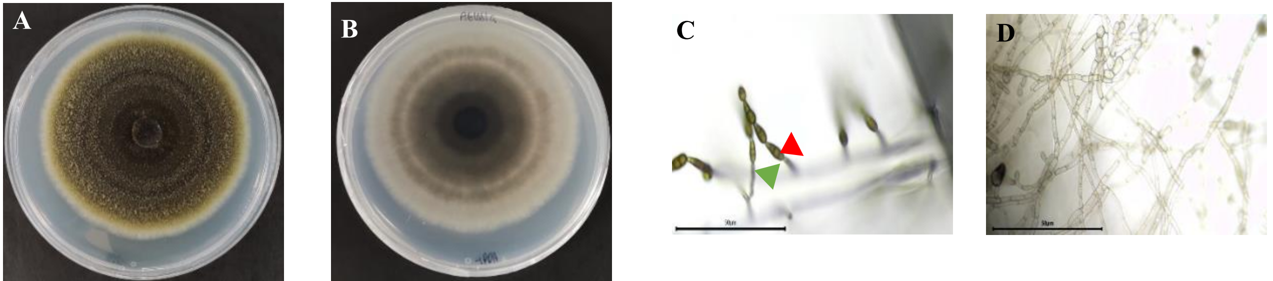
AG5



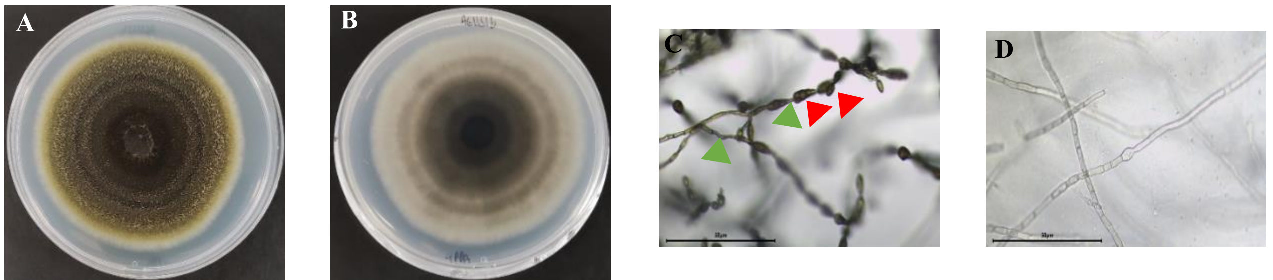
AG9S1



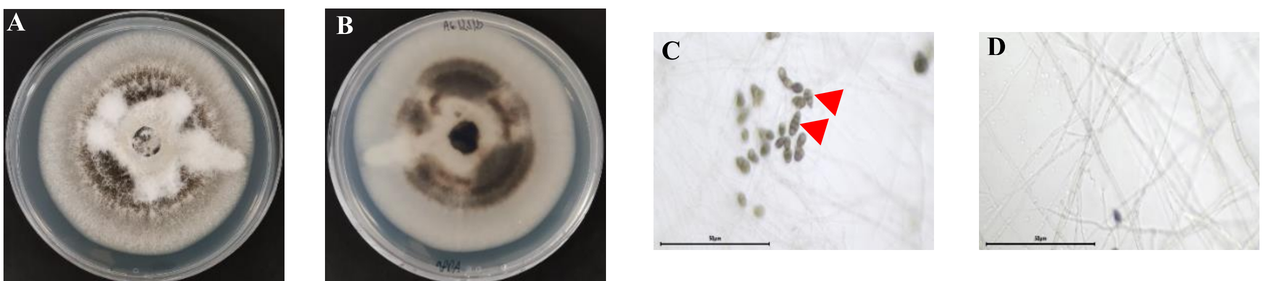
AG10S1



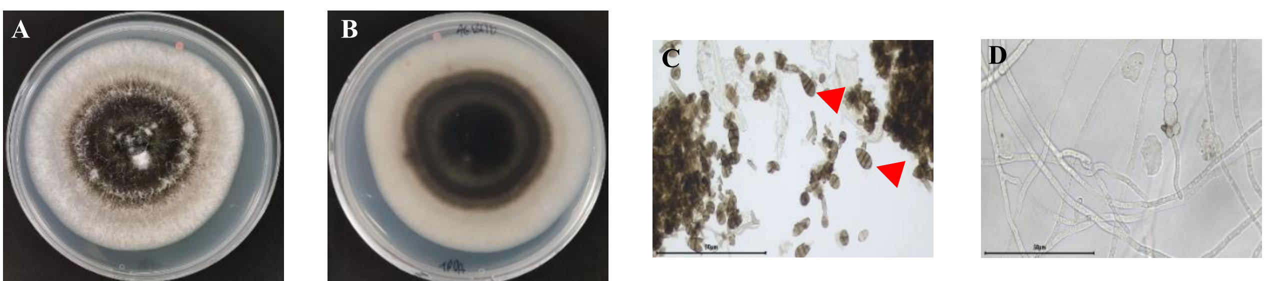
AG11S1



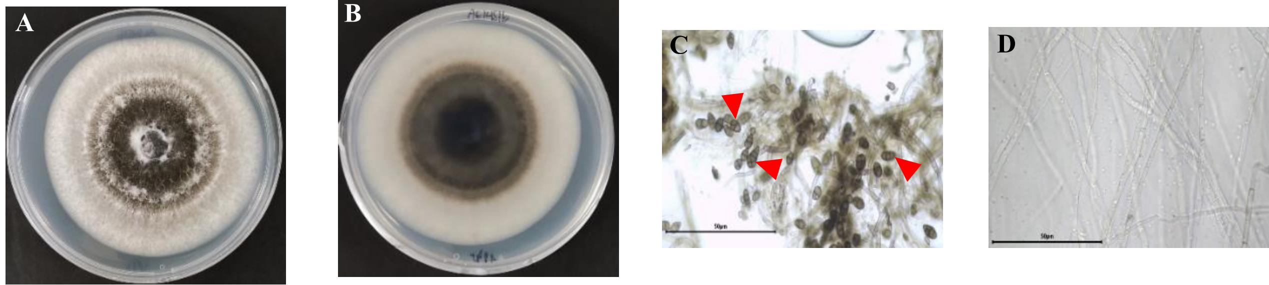
AG12S1



AG13S1



AG14S1



AON5S1

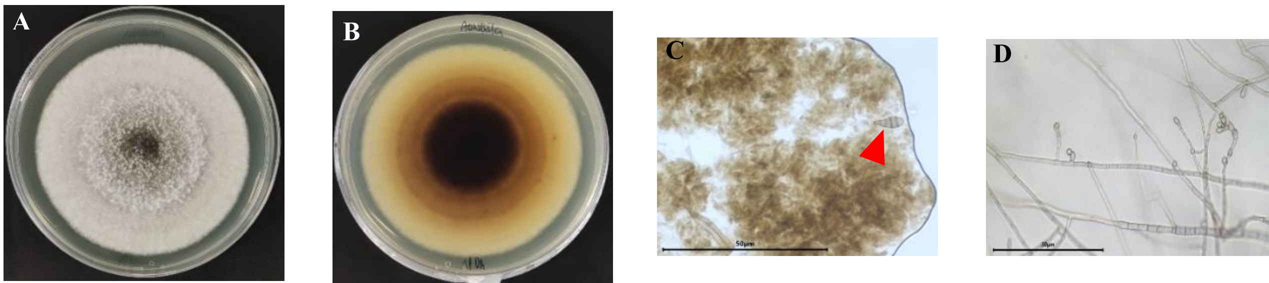


Figure 2: ***A. alternata*** **collected Northern Philippines.** The isolate was grown in qPDA and observations were conducted after seven days of incubation in dark at room temperature. (A) observe colony morphology (front) (B) reverse colony morphology (back) (C) Conidia. Red arrowheads indicate conidia and green arrow indicate conidiophore (D) Hyphae. Scale bar = 50µm.

A. alternata| GCA_016097525.1|US
A. alternata|GCA_014154925.1|CHN
A. alternata|GCA_004154755.1|UK
A. alternata|GCA_020449105.1|CHN
A. alternata|GCA_011420255.1|SK
A. alternata|GCA_009650635.1|SK
A. alternata|GCA_004634295.1|US
A. alternata|GCA_009932595.1|JPN
A. alternata|GCA_018104255.1|CHN
A. alternata|GCA_011420445.1|IND
A. alternata|GCA_011420565.1|IND
A. alternata|GCA_020085065.1|CHN
87 *A. alternata*|GCA_014751505.1|CHN
A. alternata|GCA_001572055.1|CHN
***A. alternata*|AG3|NP**
***A. alternata*|AG5|NP**
***A. alternata*|AG9S1|NP**
***A. alternata*|AG10S1|NP**
***A. alternata*|AG11S1|NP**
***A. alternata*|AG12S1|NP**
***A. alternata*|AG13S1|NP**
***A. alternata*|AG14S1|NP**
***A. alternata*|ALT1|NP**
***A. alternata*|AON5S1|NP**
***A. alternata*|ALT2|NP**
A. alternata|GCA_001696825.1|SA
A. alternata| GCA_001443195.1| US
A. radicana|AF397045.1|NTH|
0.20

Figure 3: Maximum likelihood tree of ***A. alternata*** **based on 287 sites of AA-specific gene.** The tree generated by Bayesian inference had a similar topology. This followed the Jukes-Cantor model as suggested as the best model of DNA substitution for the alignment. The tree is rooted on *A. radicana*. The highlighted isolates in red are samples collected in Philippines. The countries where the isolates were collected are indicated. US (United States), CHN (China), UK (United Kingdom), SK (South Korea), JPN (Japan), IND (India), NTH (Netherlands), and NP (Northern Philippines). Values on nodes represent percentage ML bootstrap support out of 500 bootstrap replicates. $\geq$ 50% are written before the backslash (/); Posterior probabilities $\geq$ 0.6 are shown after the backslash (/). Scale bar represents 20 nucleotide substitution every 100 nucleotides.

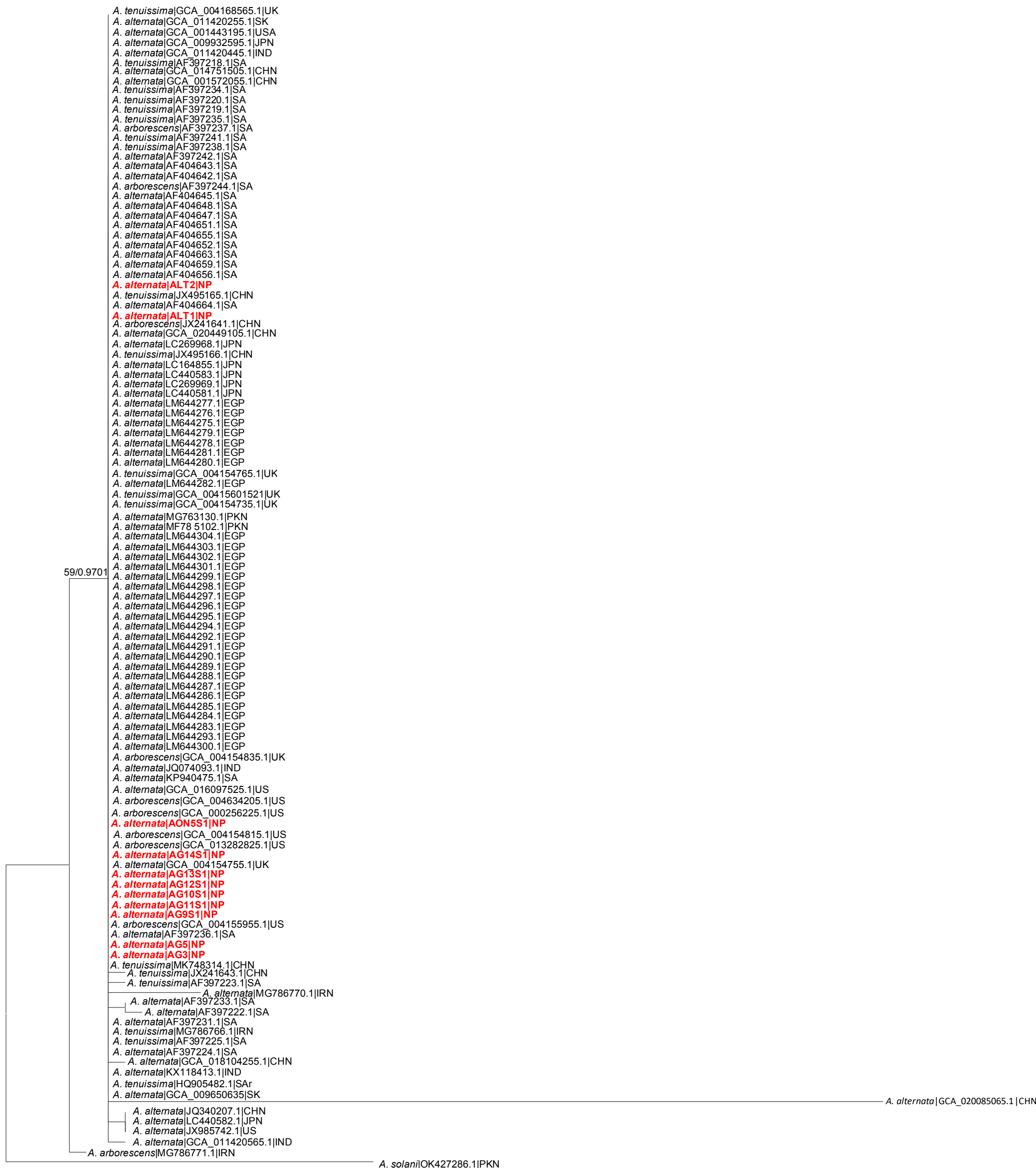


Figure 4: Maximum likelihood tree of *A. alternata* based on 442 sites of ITS gene. This followed the Jukes-Cantor model as suggested as the best model of DNA substitution for the alignment. The tree is rooted on *A. solani*. The highlighted isolates in red are samples collected in Philippines. The countries where the isolates were collected are indicated. US (United States), CHN (China), UK (United Kingdom), SK (South Korea), JPN (Japan), IND (India), SA (South Africa), EGP (Egypt), PKN (Pakistan), IRN (Iran), SAr (Saudi Arabia), and NP (Northern Philippines). Values on nodes represent percentage ML bootstrap support out of 500 bootstrap replicates. $\geq$ 50% are written before the backslash (/); Posterior probabilities $\geq$ 0.6 are shown after the backslash (/). Scale bar represents 5 nucleotide substitution every 100 nucleotides.

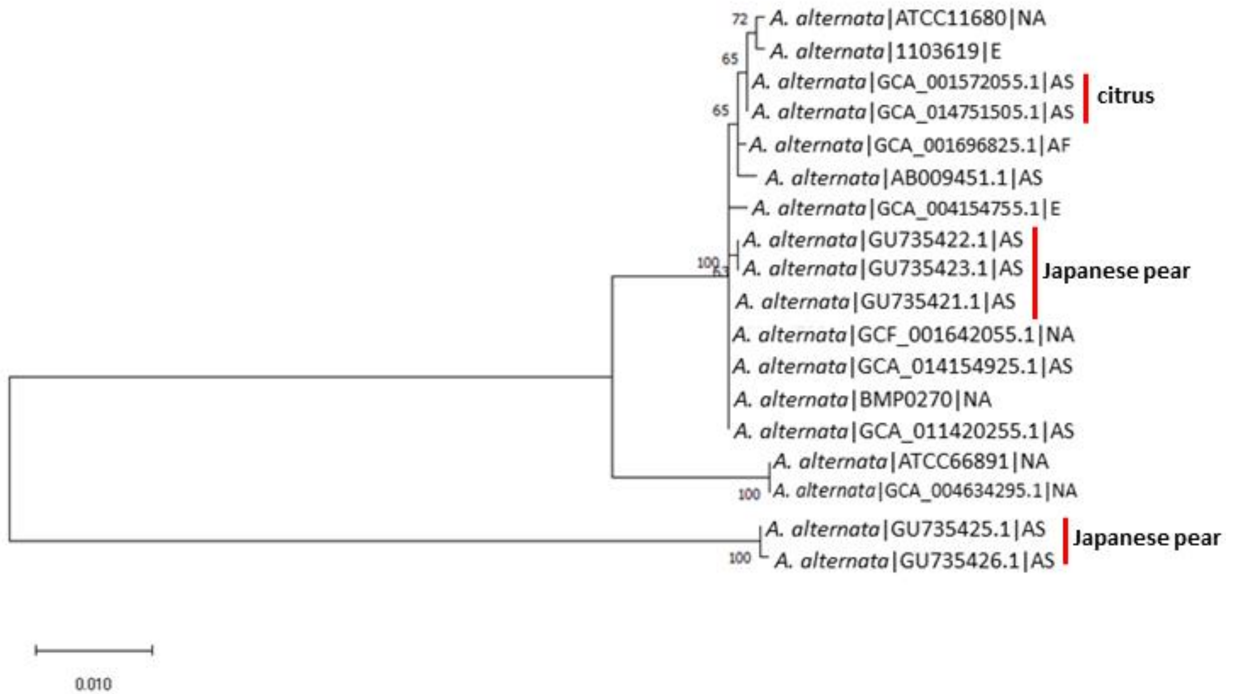


Figure 5: Maximum likelihood tree of *A. alternata* based on 760 sites of mating type 1 (1-1) gene. The values on the nodes represent bootstrap values based on 500 bootstrapped replicates generated in MEGA. The host of origin of some isolates was indicated in bold. The regions where the isolates were collected are indicated. AS (Asia), NA (North America), E (Europe), and AF (Africa). Scale bar represents 1 nucleotide substitution every 100 nucleotides.

A. tenuissima|KY559284.1|TUR
A. alternata|GCA_020449105.1|CHN
A. alternata|GCA_009650635|SK
A. alternata|KY559285.1|TUR
A. tenuissima|GCA_004154745.1|UK
A. alternata|GCA_001443195.1|US
99/1.00
A. tenuissima|GCA_017589455.1|IND
A. alternata|GCA_018104255.1|CHN
A. tenuissima|GCA_004154845.1|UK
A. tenuissima|GCA_004168565.1|UK
100/1.00
A. tenuissima|GCA_00415601521.1|UK
A. alternata|GCA_009932595.1|JPN
94/0.9981
***A. alternata*|ALT2|NP**
***A. alternata*|ALT1|NP**
A. alternata|GCA_016097525.1|US
A. alternata|GCA_011420565.1|IND
100
A. alternata|GCA_011420445.1|IND
A. arborescens|GCA_004154835.1|UK
A. arborescens|GCA_004634205.1|US
76
A. arborescens|GCA_004154815.1|US
***A. alternata*|AG14S1|NP**
81
***A. alternata*|AG13S1|NP**
***A. alternata*|AG12S1|NP**
***A. alternata*|AG11S1|NP**
98/0.6032
***A. alternata*|AG10S1|NP**
***A. alternata*|AG5|NP**
***A. alternata*|AG3|NP**
A. arborescens|GCA_000256225.1|US
***A. alternata*|AG9S1|NP**
A. solani|AB444197.1|JPN

0.50

Figure 6: Maximum likelihood tree of *A. alternata* based on 760 sites of mating type 2 (1-2) gene. The tree generated by Bayesian inference had a similar topology. This followed the Kimura-2-parameter model with invariance as suggested as the best model of DNA substitution for the alignment. The tree is rooted on *A. solani*. The highlighted isolates in red are samples collected in Philippines. The countries where the isolates were collected are indicated. US (United States), CHN (China), UK (United Kingdom), SK (South Korea), JPN (Japan), IND (India), TUR (Turkey), and NP (Northern Philippines). Values on nodes represent percentage ML bootstrap support out of 500 bootstrap replicates. $\geq$ 50% are written before the backslash (/); Posterior probabilities $\geq$ 0.6 are shown after the backslash (/). Scale bar represents 5 nucleotide substitution every 100 nucleotides.

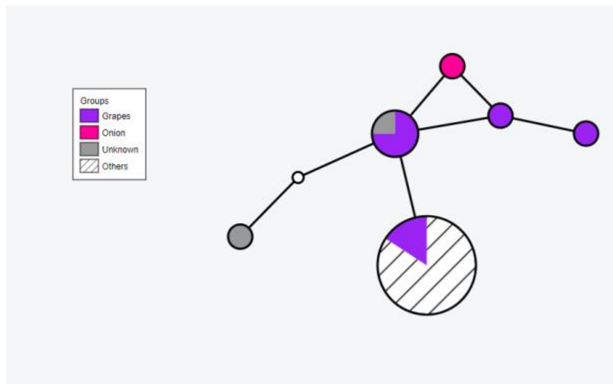


Figure 7: Parsimony network for *Alternaria alternata* populations from Northern Philippines and whole genome sequences from NCBI based on AA-specific gene constructed using TCS. The number in each circle indicates the number of individuals that shared each haplotype. The color shows at what host the sample was isolated and dots in haplotypes depict the samples isolated in Northern Philippines.

Mating-type genes in *Alternaria* species

All 11 *A. alternata* isolates were then identified whether each possesses a mating type idiomorph of either *MAT1-1* or *MAT1-2*. A PCR-based assay based on expected size, *MAT1-1* to have around 640 bp while *MAT1-2* to have around 880 bp, indicated that all local isolates from grapes and those from UPLB have *MAT1-2* (Figure 9). On the other hand, the onion isolate, AON5S1, did not produce any band for *MAT1-2* and instead showed lightly pigmented band with size indicative for amplifying *MAT1-1* . However, the mating-type amplicon of AON5S1 isolate was not included in the sequencing and the subsequent analyses due to insufficiency for further testing, despite multiple attempts for amplifying either of the mating-type alleles.

Isolates identified to possess *MAT1-2* were then sent off for sequencing to confirm the detection of the mating type idiomorph. The resulting sequences were then compared to the *MAT1-2* of isolates mined from NCBI. The sequences were aligned, consisting of thirty sequences and one outgroup creating 760 nucleotides (Table 2). ML tree constructed for *MAT 1-2* loci showed a well-supporte a ad bootstrap and posterior probabilities in each clade. This tree topology shows that *A. tenuissima* and *A. arborescens* showed a high similarity in *MAT1-2* (Figure 6). Genetic and haplotype diversity between the *A. alternata* isolates focusing on *MAT1-2* locus, showed a relatively high values of ($h = 11$; $hD = 0.867$; $S = 194$; $P = 201$; $Qw = 48.561$and $p = 0.04278$.

Other isolates from NCBI with whole genome sequences detected to possess *MAT1-1* were also compared through ML phylogenetic tree construction. The results showed that the two isolates from Europe of different host origins belonged in two different clusters in well-supported clades. The apple isolate remained a separate branch, while the *Arabidopsis thaliana* isolate was further grouped with an isolate that has unknown origin from North America with a bootstrap support value of 72. Some isolates from Asia (red), despite being taken from the exact host origin, Japanese pear (*Pyrus pyrifolia*), and the exact geographic location of Japan, belonged to separate clusters. Isolates GU735425.1 and GU735426.1 with a bootstrap support value of 63 located at the bottom-most part of the tree had the most significant difference among those taken from Japanese pear. These two sequences had a significantly different cluster from the rest of the *MAT1-1* isolates (Figure 5).

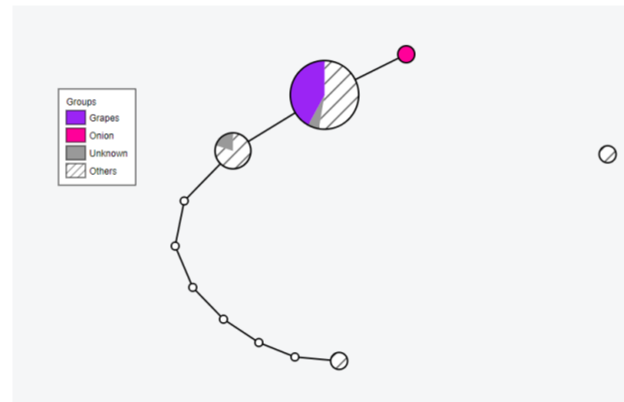


Figure 8: Parsimony network for *Alternaria alternata* populations from Northern Philippines and whole genome sequences from NCBI based on ITS gene constructed using TCS. The number in each circle indicates the number of individuals that shared each haplotype. The color shows at what host the sample was isolated and dots in haplotypes depict the samples isolated in Northern Philippines.

DISCUSSION

Alternaria alternata is a ubiquitous fungus frequently occurring as a plant pathogen of various hosts, including economically grown crops. In this study, we have detected *A. alternata* in postharvest produce, described the cultural and morphological characteristics of the isolates, and then assessed the phylogenetic relationships among different populations. Representative *A. alternata* isolates with whole genome assemblies in the database were also used for comparison. Furthermore, the mating type idiomorphs were determined to help provide insights into the possible role of mating systems in the epidemiological trajectory and another evolutionary potential of the pathogen.

A. alternata is commonly detected in various postharvest crops in different geographic locations. This pathogen is present in postharvest grapes, which causes bunch/ fruit rots. Different geographical locations were able to isolate and characterize *A. alternata* in grapes, such as in Asia, South America, and Europe, which must be monitored as it can hinder grape production (Ghuffar *et al*., 2018; Abdullah, Mahmoud, & Al-Harethi, 2016; Stocco *et al*., 2019; Lorenzini & Zapparoli, 2014). Regarding postharvest onion, Nigeria isolated *A. alternata*, which was identified to cause onion bulb rots (Samuel & Ifeanyi, 2015). In the Philippines, while commonly observed to cause deterioration in grapes, this study will be the first record of the detection *of A. alternata* on grapes and onion postharvest. Previous local studies indicate that this fungal pathogen has the potential for biocontrol of different weeds that cause problems in paddy rice, such as *Sphenoclea zeylanica* (Masangkay, 1999). However, this isolate was neither obtained from grapes or onion nor detected in any postharvest crops. It is critical to compare these isolates, especially with variations observed between isolates of the same species as this study and other literature evidenced in genetic and phenotypic characteristics. Furthermore, based on the occurrence of *A. alternata* in different locations, understanding the intraspecies variation of this fungal pathogen is critical in providing effective management methods in inhibiting the adverse effects of *A. alternata* on postharvest crops. Different management techniques are being implemented commonly by setting specific storage temperature and humidity combinations, applying growth retardants, and using chemical fungicides, which are heavily regulated (Kiaya, 2014). Recent studies have shown reduced effectivity as recorded fungal pathogens resist chemical fungicides (Kumar & Iqbal, 2020; Mari *et al.,* 2014). Alternative methods are being developed to help alleviate problems with postharvest fungal pathogens. Such as using plant extracts, essential oils, biofungicides, and nanoparticles to lessen the usage of synthetic fungicides that can

further cause fungal pathogens to evolve to be resistant (Sonker *et* al., 2016; Kumar *et al.*, 2015).

Eleven isolates of *A. alternata* in the Northern Philippines showed variable colony and morphological characteristics. The sudden decrease in rate can link to consuming both nutrients and space in the media. Despite variations in the culture colony and morphology, all of these characteristics exhibited by our local isolates correspond to *A. alternata* as previously described in other studies (Lawrence *et al.,* 2016; Woudenberg *et al.,* 2013; Meena & Samal, 2019). As observed in the colony characteristics of isolated *A. alternata* in Northern Philippines (Fig. 2), the isolates collected from two (2) different hosts and within the populations as observed to samples collected from one host (grapes) have been observed to exhibit variations despite being exposed to similar conditions. Similar observations of the varied colony and morphology characteristics among the populations of *A. alternata* isolated from tomatoes in Algeria were detected (Bessadat *et al.,* 2014). Another possible reason for this is that some of the *A. alternata* isolates have undergone mutation, migration, and cryptic recombination events, which leads to variation within the population, as observed in *A. alternata* isolated from sunflower (Kgatle *et al.,* 2018). Conidia, in this study, manifest two different characteristics. Some isolates exhibited chained conidia, and others showed single conidia. Detected *A. alternata* from various hosts (blueberry, potato, and grapes) expresses a conidial chain characteristic. After the pathogenicity test, it was observed that all isolates that manifested this feature could infect their hosts (Zhu & Xiao, 2015; Zheng *et al.,* 2015; Tymon *et al.,* 2016; Lorenzini & Zapparoli, 2014). Despite numerous attempts to promote sporulation, some isolates did not manifest conidial chain phenotype, possibly attributed to intraspecies variations.

Furthermore, molecular confirmation of isolate ID was verified using *AA-specific* primers for *A. alternata*, also used to identify different *A. alternata* from various plant host and geographic locations such as carob, tomato, wheat, malt, rice, bean, mangrove, fig, and potato (Basim *et al*., 2018; Sirma *et al*., 2021; Meena *et al*., 2016; Kamal, 2015; Lin *et al*., 2015; Latinovic *et al.,* 2014; Dube *et al.,* 2014). *A. alternata* are widely distributed and cause plant diseases. This can also be applied to the isolates and the sequences retrieved from NCBI as the hosts. The geographic location of representative *A. alternata* in this study did not seem to factor in the formation of clades in the constructed phylogenetic trees. The ITS locus classified the three *Alternaria* species into one clade: *A. alternata*, *A. tenuissima*, and *A. arborescens*. *Alternaria* species are challenging to delineate as morphological features are not suitable to describe the evolutionary relationships occurring in *Alternaria* species. The two former species are found to be possibly represented under the same species group with *A. alternata* (Armitage *et al*., 2015). Molecular-based identification and classification have been widely used to provide information on the relationships of different species being studied. The internal transcribed spacer (ITS) region is considered the primary gene locus for molecular analysis of fungal species. However, it was identified that this locus might not be variable in all fungi groups, especially in *Ascomycota*. Focusing on the result of phylogenetic trees on Genus *Alternaria*, morphological verification was performed due to sequence similarities of *A. infectoria* and *A. alternata.* It also indicated that those additional loci must be used as ITS can no longer distinguish the two species by this locus alone (Baturo-Ciesniewska *et al.,* 2020). This dramatically reflects the results of constructed phylogenetic tree based on ITS wherein two *Alternaria* species formed alongside *A. alternata* in a single clade. It is good to note that the AA-specific primer used in this study was based on restriction fragment length polymorphism (RFLP) analysis of the ITS region of different *Alternaria* species (Konstantinova *et al*., 2002). Ideally, as AA-specific is derived from ITS, the result is to be expected. It is recommended to use additional loci to classify Alternaria species accurately. The total genetic and haplotype diversity for the ITS and AA-specific were low. The parsimony network supported this as it expressed that the representative populations did not undergo recombination based on ITS locus. Our results also somehow resemble the study of ***Alternaria* spp. strains that** cause grape bunch rot wherein a phylogenetic tree based on ITS gene, *A. alternata, A tenuissima,* and *A. arborescens* formed a monophyletic clade that is well supported by a high bootstrap value (Lorenzini & Zapparoli, 2014). These results support that using the ITS gene as a single locus will not be suitable enough to provide accurate diversity with *Alternaria* spp. However, in this study, it is recommended to collect more isolates, and using hypervariable loci may provide more information for a fine-scale population assessment. In this study, the total values were accounted for and did not conduct computation in each host due to the slight and high variance of host sources (Rozas, 2017). This contrasting result of the estimated values and the actual haplotype network is due to their calculation exclusivity. In DnaSP, if the alignment contains gaps, this will be excluded in the computation, while in TCS, gaps are included resulting in the observed network.

A. alternata is known to reproduce asexually strictly. In the study, each of the local *A. alternata* isolates sampled in Northern Philippines were demonstrated to possess only one mating type gene indicative of a heterothallic or a self-incompatible fungus. A certain fungus that is said to be heterothallic or self-incompatible indicate individuals to possess only one mating type allele/idiomorph, where expected mating would require two compatible partners, each bearing a different idiomorph, e.g., one individual with *MAT1-1*, the other with *MAT1-2* (Ni *et al.,* 2011). *A. alternata* sampled populations in China revealed isolates to be heterothallic with each individual carrying one mating type. While the detection of *MAT* idiomorphs could already provide indications for sexual reproduction, with *MAT* genes known in regulating sex in other fungi, a 1:1 ratio of mating type frequencies (occurrence of 1:1 *MAT1-1* and *MAT1-2* in a population) or getting close to this ratio would indicate higher chances for random mating in a population. This implies that our results provide support that the local isolates are heterothallic because each isolate carries only one MAT allele (*MAT1-2*) but expected mating cannot be met due to lack of random mating where 1:1 ratio of mating type idiomorphs frequencies hasn't been detected. Occurrence was in equal frequencies of a 1:1 ratio of *MAT1-1* and *MAT1-2* genes. Another study in Turkey characterized *A. alternata* from *Pistacia* spp. and determined that *A. alternata* population from the host had an equal frequency of both idiomorphs *MAT1-1* and *MAT1-2* at different geographical locations. Both mating type idiomorphs in equal frequencies in a population would provide evidence for sexual reproduction. In this study in Japan, it was found that the isolates were found to have a 1:1 *MAT1-1* to *MAT1-2* ratio. It was observed that six isolates were carrying *MAT1-1,* and six isolates were carrying *MAT1-2*. A 1:1 mating type distribution ratio is indirect evidence of sexual reproduction because of frequency dependency (Ozkilinc *et al.,* 2017; Meng *et al.,* 2015). When there is an equal mating type distribution, there is a higher chance of random mating occurring (Milgroom, 1996). The results show a 0.6 occurrence of *MAT1-1* and 0.4 occurrences of *MAT1-2*. These results supported our findings wherein only *MAT1-2* (Figure 9) was detected in all isolates from grape and those with an unknown host (Table 2). Supposedly, an equal ratio of the mating type frequency across all the isolates collected in a specific population suggests a balancing selection where two or more alleles exist in the population's gene pool. This mostly applies to the local population, but the implications of a 1:1 ratio among a wider geographic range must be further studied. The gene pool during a balancing selection could be maintained through sexual reproduction where there is a constant contribution of different alleles in the population, causing high allelic diversity. Balancing selection for asexual organisms is relatively low since this type of reproduction

Figure 9: Agarose gel photograph of amplicons of *A. alternata* in Northern Philippines showing the presence or absence of MAT 1-2 loci.

Table 2: List of *Alternaria alternata* isolates collected from different hosts in the Northern Philippines

Species	Host	Mating type	Location
Alt1	Unknown	Mat 1-2	University of the Philippines-Los Baños
Alt2	Unknown	Mat 1-2	University of the Philippines-Los Baños
AG3	*Vitis* sp.	Mat 1-2	Local Market (Baguio City)
AG5	*Vitis* sp.	Mat 1-2	Local Market (Baguio City)
AG9S1	*Vitis* sp.	Mat 1-2	Local Market (Baguio City)
AG10S1	*Vitis* sp.	Mat 1-2	Local Market (Baguio City)
AG11S1	*Vitis* sp.	Mat 1-2	Local Market (Baguio City)
AG12S1	*Vitis* sp.	Mat 1-2	Local Market (Baguio City)
AG13S1	*Vitis* sp.	Mat 1-2	Local Market (Baguio City)
AG14S1	*Vitis* sp.	Mat 1-2	Local Market (Baguio City)
AON5S1	*Allium cepa*	Mat 1-1	Nueva Ecija
A. alternata JS-1623	*Abies koreana*	Mat 1-2	South Korea: Gyeongbuk
A. alternata PN1	*Brassica juncea*	Mat 1-2	India
A. alternata PN2	*Brassica juncea*	Mat 1-2	India
A. alternata ATCC 34957	*Sorghum bicolor*	Mat 1-2	USA: Kansas
A. alternata hznu325	*Chrysanthemum*	Mat 1-2	China: Tongxiang
A. alternata PF1	Plastic debris	Mat 1-2	China: Qingdao
A. alternata EV-MIL-31	Citrus (Minneola tangelo)	Mat 1-2	Florida
A. alternata NAP07	*Prunus persica*	Mat 1-2	Japan
A. alternata Z7	*Citrus reticulata*	Mat 1-1	China: Zheijiang
A. alternata Y784-BC03	Hongyang kiwifruit	Mat 1-1	China: Chongging
A. alternata JS-0527	*Phragmites australis*	Mat 1-1	South Korea: Gyeongbuk
A. alternata B3	*Ophiopogon japonicus*	Mat 1-1	China: Zhejiang
A. alternata Z7	*Citrus suavissima*	Mat 1-1	China: Wenzhou
A. alternata FERA 1177	*Malus domestica*	Mat 1-1	United Kingdom
A. alternata B2a	*Allium cepa*	Mat 1-1	South Africa: Pretoria-Roodeplaat
A. alternata MOD1-FUNGI5	*Vitis* sp. (Seedless)	Mat 1-1	USA:MD
A. tenuissima FERA 648	*Pyrus* sp.	Mat 1-2	United Kingdom
A. tenuissima ANJ	Garden soil	Mat 1-2	India: Durgapur
A. tenuissima FERA 1164	*Malus domestica*	Mat 1-2	United Kingdom
A. tenuissima FERA 24350	*Pyrus pyrifolia*	Mat 1-2	United Kingdom

Species	Host	Mating type	Location
A. tenuissima FERA 743	*Malus domestica*	Mat 1-2	United Kingdom
A. tenuissima FERA 1082	*Malus domestica*	Mat 1-2	United Kingdom
A. tenuissima FERA 635	*Malus domestica*	Mat 1-1	United Kingdom
A. arborescens FERA 675	*Pyrus pyrifolia*	Mat 1-2	United Kingdom
A. arborescens EGS 39-128	*Solanum lycopersicum*	Mat 1-2	California
A. arborescens RGR 97.0013	*Malus domestica*	Mat 1-1	USA (USDA)
A. arborescens RGR 97.0016	*Malus domestica*	Mat 1-2	USA (USDA)
A. arborescens MOD1-FUNGI6	Red apple	Mat 1-2	USA:MD
A. alternata	*Pistachia* sp.	Mat 1-2	Turkey
A. tenuissima	*Pistachia* sp.	Mat 1-2	Turkey
A. arborescens	Tomato	Mat 1-2	Turkey

does not necessarily lead to diversity (Navascués *et al.,* 2010; Siewert & Voight, 2017).

Besides *A. alternata, A. tenuissima and A. solani* populations were also reported to have high genetic variation suggesting the occurrence of cryptic sexual reproduction (Yang *et al.,* 2018). Due to the small number of isolates collected in this study, it is recommended to conduct more sampling and isolation of *A. alternata* to provide a better probability for sexual reproduction and to identify the mating potential of *A. alternata*. It was previously mentioned that *A. alternata* has already been detected and characterized in grapes from different geographic locations. Identification for the presence of mating type idiomorphs was not included in the characterization of *A. alternata*. Other literature for *A. alternata* in grapes is more on detection, pathogenicity and mycotoxins, and different management strategies (Mikusova & Srobarova, 2014; Ghuffar *et al.,* 2018; Prendes *et al.,* 2015; Lorenzini & Zapparoli, 2014). Although a faded band, the isolate from onion AON5S1 had shown an indication of the presence of *MAT1-1*. Despite multiple attempts to produce a better amplicon for *MAT1-1,* the researcher could not improve the produced amplicon (figure did not show). This result can be linked to different reasons, such as the template DNA use is low in concentration, and the components in the master mix were not sufficient enough to produce an evident amplicon. Evidence showed that there was a high level of genotypic diversity, low non-random associations within SSR markers in the populations, an equal level of mating types, and low genetic differentiation depicting that sexual recombination of *A. alternata* had occurred in the field population (Meng *et al.,* 2015). In this study, the representative isolates were identified to only have either *MAT1-1* or *MAT1-2* mating types (Table 2). This initial result can provide information that *A. alternata* is not confined to asexual reproduction only. However, in nature occurrence of sexual reproduction in *A. alternata* is not yet observed. Phylogenetic trees based on *MAT1-2* also depicted a low derivation among *A. alternata, A. tenuissima,* and *A. arborescens,* indicating that *A. alternata* can suggest that cross-recombination is possible within *Alternaria* spp. However, in contrast to the other two loci, we can see that plant host origin can be a factor in the formation of different clades in this phylogenetic tree, as shown by the *MAT 1-1* phylogenetic tree. The detection of *MAT 1-2* in these isolates and the comparison with the *MAT 1-2* of other Alternaria species show a signature for cryptic compatibility between three species. *Alternaria* species can produce toxins that are classified into two: non-host selective toxins (nHST) and host selective toxins (HST) (Tsuge *et al*., 2013; Meena *et al*., 2017). *A. alternata* is identified as the pathogen responsible for three different plant diseases in citrus such as Alternaria brown spot in tangerines, Alternaria leaf spot of rough lemon, and Alternaria black rot of other citrus fruits are only differentiated by the toxin it released that can only be identified by conducting pathogenicity test, toxin assay or genetic markers (Akimitsu, Peever, & Timmer, 2003). Different fungal pathogens transition to dormancy when in storage but then breaks dormancy once conditions are conducive. For instance, in terms of fruit production, postharvest pathogens typically infect harvested fruit via breaching the host cuticle and will remain unnoticed for months until the harvested fruit ripens (Alkan & Fortes, 2015). Some fungi can remain in storage because of their saprophytic nature and can be influenced by biotic and abiotic factors that promote postharvest disease development (Prusky *et al.,* 2013). The fungus may utilize asexual reproduction to generate millions of spores (Sun *et al*., 2020). On the other hand, the fungal population may undergo sexual reproduction and generate novel genotypes through recombination. A sexual reproduction cycle in nature may not have been observed for *A. alternata*. The detected presence of a single conserved mating type gene in all isolates in this study can indicate that recombination is occurring. The same observation was documented for *P. digitatum* (Marcet-Houben *et al.,* 2012). These novel genotypes may confer virulence or pathogenicity, leading to fungicide resistance, host jumps, or the production of host selective toxins to better adapt to new environments.

The detection of *A. alternata* isolates in postharvest produce and the subsequent characterization through morphological and sequence analyses provide insights into the underlying basis of variations in the biology within this species of various hosts. Despite being unable to test for symptomatology and perform infection studies, detecting the *A. alternata* isolates in postharvest produce is critical in disease management. For instance, appropriate strategies can be implemented when handling fresh produce in storage to avoid food spoilage and wastage. Furthermore, this study aids in understanding factors involved in the epidemiology of the disease, particularly associated with critical steps in the disease cycle. Knowledge of the morphological and genetic characteristics can help further to determine sources of inoculum and modes of pathogen dispersal.

ACKNOWLEDGMENT

The authors would like to acknowledge the Natural Sciences Research Institute for providing financial support with project code BIO-20-1-01 during the rendering of this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest between the manuscript material and any financial, personal, or other relationship with other people or organizations.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

The first author conducted the implementation of methods and analyses of the study and the subsequent drafting of the manuscript. The second author contributed to performing some of the *in silico* analyses and the subsequent manuscript drafting. The third or the corresponding author provided conception, study design, and overall supervision of the study, as well as a review of the manuscript.

REFERENCES

Abdullah, Q., Mahmoud, A., & Al-Harethi, A. (2016). Isolation and identification of fungal post-harvest rot of some fruits in Yemen. *PSM Microbiol*, *1*(1), 36-44.

Akimitsu, K., Peever, T. L., & Timmer, L. W. (2003). Molecular, Ecological, and Evolutionary Approaches to Understanding *Alternaria* Diseases of Citrus. *Molecular Plant Pathology*, *4*(6), 435-446.

Alkan, N., & Fortes, A. M. (2015). Insights into Molecular and Metabolic Events Associated with Fruit Response to Post-harvest Fungal Pathogens. *Frontiers in Plant Science*, *6*, 889.

Almeida, F., Rodrigues, M. L., & Coelho, C. (2019). The still underestimated problem of fungal diseases worldwide. *Frontiers in Microbiology*, 214.

Alvindia, D. G., & de Guzman, M. F. (2016). Survey of Philippine coffee beans for the presence of ochratoxigenic fungi. *Mycotoxin Research*, *32*(2), 61-67.

Balendres, M. A. O., Karlovsky, P., & Cumagun, C. J. R. (2019). Mycotoxigenic fungi and mycotoxins in agricultural crop commodities in the Philippines: A review. *Foods*, *8*(7), 249.

Basım, H., Basım, E., Baki, D., Abdulai, M., Öztürk, N., & Balkic, R. (2018). Identification and characterization of *Alternaria alternata* (Fr.) Keissler causing Ceratonia Blight disease of carob (*Ceratonia siliqua L.*) in Turkey. *European Journal of Plant Pathology*, *151*(1), 73-86.

Baturo-Cieśniewska, A., Pusz, W., & Patejuk, K. (2020). Problems, limitations, and challenges in species identification of *Ascomycota* members on the basis of ITS regions. *Acta Mycologica*, *55*(1).

Bessadat, N., Simoneau, P., Benichou, S., Setti, B., Kihal, M., & Henni, D. E. (2014). Morphological, physiological, and pathogenic variability of small-spore Alternaria sp. causing leaf blight of Solanaceous plants in Algeria. *African Journal of Microbiology Research*, *8*(37), 3422-3434.

Chen, Y., Zhang, Z., Tian, S., & Li, B. (2022). Application of-omic technologies in postharvest pathology: Recent advances and perspectives. *Current Opinion in Food Science*, *45*, 100820.

Cueva, F. D., Mendoza, J. S., & Balendres, M. A. (2018). A new Colletotrichum species causing anthracnose of chili in the Philippines and its pathogenicity to chili cultivar Django. *Crop Protection*, *112*, 264-268.

Cruz, A. et.al., (2013). Phylogenetic Analysis, Fumonisin Production and Pathogenicity of *Fusarium fujikuroi* Strains Isolated from Rice in the Philippines. *Journal of the Science of Food and Agriculture*, *93*(12), 3032-3039.

Dube, J. P., Truter, M., & Van Der Waals, J. E. (2014). First report of resistance to QoI fungicides in Alternaria alternata isolates from potato in South Africa. *Plant Disease*, *98*(10), 1431-1431.

Fernández, J., Rivera-Vargas, L. I., Cabrera-Asencio, I., & Cantrell, S. A. (2011). Alternaria spp. implicated in a disease complex of onion leaf blight in the tropics. *The Journal of Agriculture of the University of Puerto Rico*, *95*(1-2), 57.

Fisher, M. C. et.al., (2012). Emerging fungal threats to animal, plant, and ecosystem health. *Nature*, *484*(7393), 186-194.

Ghuffar, S., Irshad, G., Shahid, M., Naz, F., Riaz, A., Khan, M. A., ... & Gleason, M. L. (2018). First report of *Alternaria alternata* causing fruit rot of grapes in Pakistan. *Plant Disease*, *102*(8), 1659-1659.

Huelsenbeck, J. P., and F. Ronquist. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*. 17:754-755.

Jain, A., Sarsaiya, S., Wu, Q., Lu, Y., & Shi, J. (2019). A review of plant leaf fungal diseases and its environment speciation. *Bioengineered*, *10*(1), 409-424.

Kamal, S. A., (2015). Detection of *Alternaria alternata* isolated from grain by using specific primer. *World Journal of Pharmaceutical Research*, *4*(7), 260-269.

Kgatle, M. G., Truter, M., Ramusi, T. M., Flett, B., & Aveling, T. A. S. (2018). *Alternaria alternata*, the causal agent of leaf blight of sunflower in South Africa. *European Journal of Plant Pathology*, *151*(3), 677-688.

Kiaya, V. (2014). Post-harvest losses and strategies to reduce them. *Technical Paper on Postharvest Losses, Action Contre la Faim (ACF)*, *25*, 1-25.

Koichiro Tamura, Glen Stecher, and Sudhir Kumar (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* 38:3022-3027

Konstantinova, P., Bonants, P. J., Van Gent-Pelzer, M. P., & Van Der Zouwen, P. (2002). Development of specific primers for detection and identification of *Alternaria* spp. in carrot material by PCR and comparison with blotter and plating assays. *Mycological Research*, *106*(1), 23-33.

Kumar, V., & Iqbal, N. (2020). Post-harvest pathogens and disease management of horticultural crop: A brief review. *Plant Arch*, *20*, 2054-2058.

Kumar, V., Neeraj, S. S., & Sagar, N. A. (2015). Post-harvest management of fungal diseases in onion—a review. *International Journal of Current Microbiology and Applied Sciences*, *4*(6), 737-52.

Latinović, N., Radišek, S., & Latinović, J. (2014). First report of *Alternaria alternata* causing fruit rot on fig (*Ficus carica*) in Montenegro. *Plant disease*, *98*(3), 424-424.

Lawrence, D. P., Rotondo, F., & Gannibal, P. B. (2016). Biodiversity and Taxonomy of the Pleomorphic Genus *Alternaria*. *Mycological Progress*, *15*(1), 3.

Lin, Q. L., Su, H. R., & He, H. (2015). First report of *Alternaria alternata* causing leaf spot on *Bruguiera gymnorrhiza* in China. *Plant Disease*, *99*(2), 286-286.

Lorenzini, M., & Zapparoli, G. (2014). Characterization and pathogenicity of *Alternaria* spp. strains associated with grape bunch rot during post-harvest withering. *International Journal of Food Microbiology*, *186*, 1-5.

Marcet-Houben, M., Ballester, A. R., de la Fuente, B., Harries, E., Marcos, J. F., González-Candelas, L., & Gabaldón, T. (2012). Genome sequence of the necrotrophic fungus *Penicillium digitatum*, the main postharvest pathogen of citrus. *BMC Genomics*, *13*(1), 1-18.

Mari, M., Di Francesco, A., & Bertolini, P. (2014). Control of fruit postharvest diseases: old issues and innovative approaches. *Stewart Postharvest Review*, *10*(1), 1-4.

Masangkay, R. F., Paulitz, T. C., Hallett, S. G., & Watson, A. K. (1999). Factors influencing biological control of *Sphenoclea zeylanica* with *Alternaria alternata* f. sp. *sphenocleae*. *Plant disease*, *83*(11), 1019-1024.

Matić, S., Tabone, G., Garibaldi, A., & Gullino, M. L. (2020). Alternaria leaf spot caused by Alternaria species: an emerging problem on ornamental plants in Italy. *Plant disease*, *104*(8), 2275-2287.

Meena, M., & Samal, S. (2019). *Alternaria* host-specific (HSTs) Toxins: An Overview of Chemical Characterization, Target Sites, Regulation and Their Toxic Effects. *Toxicology Reports*, *6*, 745-758.

Meena, M. et.al., (2017). *Alternaria* Toxins: Potential virulence factors and genes related to pathogenesis. *Frontiers in Microbiology*, *8*, 1451.

Meena, M., Zehra, A., Dubey, M. K., Aamir, M., Gupta, V. K., & Upadhyay, R. S. (2016). Comparative evaluation of biochemical changes in tomato (*Lycopersicon esculentum* Mill.) infected by *Alternaria alternata* and its toxic metabolites (TeA, AOH, and AME). *Frontiers in plant science*, *7*, 1408.

Meng, J. W. et.al., (2015). Population Genetic Analysis Reveals Cryptic Sex in the Phytopathogenic Fungus *Alternaria alternata*. *Scientific Reports*, *5*(1), 1-10.

Mikušová, P., Sulyok, M., & Šrobárová, A. (2014). *Alternaria* mycotoxins associated with grape berries in vitro and in situ. *Biologia*, *69*(2), 173-177.

Milgroom, M. G. (1996). Recombination and the multilocus structure of fungal populations. *Annual Review of Phytopathology,* *34*(1), 457–477. doi: 10.1146/annurev.phyto.34.1.457

Múrias dos Santos, A., Cabezas, M. P., Tavares, A. I., Xavier, R., & Branco, M. (2016). tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics*, *32*(4), 627-628.

Navascués, M., Stoeckel, S. & Mariette, S (2010). Genetic diversity and fitness in small populations of partially asexual, self-incompatible plants. *Heredity* 104, 482–492. https://doi.org/10.1038/hdy.2009.159

Ni, M., Feretzaki, M., Sun, S., Wang, X., & Heitman, J. (2011). Sex in fungi. *Annual review of genetics*, *45*, 405.

Ozkilinc, H., Sarpkaya, K., Kurt, S., Can, C., Polatbilek, H., Yasar, A., ... & Konukoglu, F. (2017). Pathogenicity, morpho-species, and mating types of *Alternaria* spp. causing *Alternaria* blight in *Pistacia* spp. in Turkey. *Phytoparasitica*, *45*(5), 719-728.

Prendes, L. P., Merín, M. G., Andreoni, M. A., Ramirez, M. L., & de Ambrosini, V. I. M. (2015). Mycobiota and toxicogenic *Alternaria* spp. strains in Malbec wine grapes from DOC San Rafael, Mendoza, Argentina. *Food Control*, *57*, 122-128.

Prusky, D., Alkan, N., Mengiste, T., & Fluhr, R. (2013). Quiescent and necrotrophic lifestyle choice during postharvest disease development. *Annual Review of Phytopathology*, *51*(1), 155-176.

Rozas, J., Ferrer-Meta, A., Sanchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramons-Onsins S.E., Sanchez-Gracia, A. (2017). DnaSP v6: DNA Sequence Polymorphism Analysis of Large Datasets *Mol. Biol. Evol.* 34: 3299-3302 (2017).

Samuel, O., & Ifeanyi, O. (2015). Fungi associated with the deterioration of post-harvest onion bulbs sold in some markets in Awka, Nigeria. *Bioengineering and Bioscience*, *3*(5), 90-94.

Santiago, N. C., Roderos, B. P., & Gallerma, F. M. (2018). Management of pre- and post-harvest diseases in bulb onion through *Trichoderma harzianum rifai* utilization. *Asian Journal of Postharvest and Mechanization*, *1*(2), 34.

Sirima, A., Sereme, A., Koita, K., Elisabeth, Z. I. D. A., Sereme, D., & Theodore, N. A. N. A., & Sawadogo, M. (2021). Characterization of *Alternaria brassicicola* isolated from tomato in Burkina Faso, and use of two essential oils for its control in vitro. *African Journal of Agricultural Research*, *17*(11), 1371-1379.

Siewert, K. M., & Voight, B. F. (2017). Detecting long-term balancing selection using allele frequency correlation. *Molecular Biology and Evolution, 34*(11), 2996–3005. doi:10.1093/molbev/msx209

Singh, D., & Sharma, R. R. (2018). Postharvest diseases of fruits and vegetables and their management. In *Postharvest Disinfection of Fruits and Vegetables* (pp. 1-52). Academic Press.

Sonker, N., Pandey, A. K., & Singh, P. (2016). Strategies to control post-harvest diseases of table grape: A review. *Journal of Wine Research*, *27*(2), 105-122.

Stewart, J. E., Thomas, K. A., Lawrence, C. B., Dang, H., Pryor, B. M., Timmer, L. M., & Peever, T. L. (2013). Signatures of recombination in clonal lineages of the citrus brown spot pathogen, *Alternaria alternata* sensu lato. *Phytopathology*, *103*(7), 741-749.

Sun, S., Hoy, M. J., & Heitman, J. (2020). Fungal pathogens. *Current Biology*, *30*(19), R1163-R1169.

Stocco, A. F., Diaz, M. E., Romera, M. R., Mercado, L. A., Rivero, M. L., & Ponsone, M. L. (2019). Biocontrol of

postharvest *Alternaria* decay in table grapes from Mendoza province. *Biological Control*, *134*, 114-122.

Troncoso-Rojas, R., & Tiznado-Hernández, M. E. (2014). *Alternaria alternata* (black rot, black spot). In *postharvest decay* (pp. 147-187). Academic Press.

Tsuge, T., et.al., (2013). Host-Selective Toxins Produced by the Plant Pathogenic Fungus *Alternaria alternata*. *FEMS Microbiology Reviews*, *37*(1), 44-66.

Tymon, L. S., Peever, T. L., & Johnson, D. A. (2016). Identification and enumeration of small-spored *Alternaria* species associated with potato in the US Northwest. *Plant disease*, *100*(2), 465-472.

Yang, N., Ma, G., Chen, K., & Wu, X. (2018). The population genetics of *Alternaria tenuissima* in four regions of China as determined by microsatellite markers obtained by transcriptome sequencing. *Frontiers in Microbiology, 9.* doi:10.3389/fmicb.2018.02904

Zheng, H. H., Zhao, J., Wang, T. Y., & Wu, X. H. (2015). Characterization of *Alternaria* species associated with potato foliar diseases in China. *Plant Pathology*, *64*(2), 425-433.

Zhu, X. Q., & Xiao, C. L. (2015). Phylogenetic, morphological, and pathogenic characterization of *Alternaria* species associated with fruit rot of blueberry in California. *Phytopathology*, *105*(12), 1555-1567.

Supplementary Table 1: Growth Rate of *Alternaria alternata* isolated from Northern Philippines

Isolate Name	COLONY SIZE (mm)					
	qPDA					
	DAY 3		DAY 5		DAY 7	
	Average	Rate	Average	Rate	Average	Rate
Alt1	44.5	7.9	60.7	0.36	77	0.26
Alt2	45.15	8.03	63.45	0.40	77.15	0.21
AG3	35.85	6.17	52.4	0.46	69.05	0.31
AG5	34	5.8	48.95	0.43	68.3	0.39
AG9S1	36.05	6.21	51.65	0.43	68.85	0.33
AG10S1	35.8	6.16	51.55	0.44	68.05	0.32
AG11S1	37.4	6.48	54.1	0.45	70.45	0.30
AG12S1	35.7	6.14	51.45	0.44	63.8	0.24
AG13S1	35.35	6.07	51.4	0.45	70.9	0.38
AG14S1	35.35	6.07	52.65	0.49	71.4	0.36
AON5S1	35.6	6.12	51.3	0.44	69.9	0.36

Supplementary Table 2: Representative *Alternaria* species isolates with whole genome sequences used in this study

GenBank Accession Number	Alternaria species	Strain	Genome Information				Host	Location	Sequence Obtained
			Total Genome Length	Assembly Level	Assembly Method	Sequencing Technology			
GCA_014751 505.1	*A. alternata*	Z7	34,280,341	Complete Genome	SPAdes v.1	Oxford Nanopore	*Citrus reticulata*	China: Zhejiang	ITS, AA-specific, Mat 1-1
GCA_020085 065.1	*A. alternata*	Y784-BC03	33,866,835	Chromosom e	ABySS V.2.1.5; pilon v.1.24	Illumina NovaSeq; Oxford Nanopore	Hongyang kiwifruit	China: Chongging	ITS, AA-specific, Mat 1-1
GCA_009932 595.1	*A. alternata*	NAP07	35,840.006	Scaffold	Canu v1.7	Nanopore MinION	*Prunus persica*	Japan	ITS, AA-specific, Mat 1-2
GCA_018104 255.1	*A. alternata*	PF1	34,859,069	Contig	Unicycler V.2.0. v. 3.0	Illumina PE150; Nanopore	Plastic debris	China: Qingdao	ITS, AA-specific, Mat 1-2
GCA_009650 635	*A. alternata*	JS-1623	33,670,787	Scaffold	FALCON v.0.3.0; SOAPdenovo v. 2.04; SSPACE	PacBio RSII; Illumina HiSeq	*Abies koreana* stem	South Korea: Gyeongbuk	ITS, AA-specific, Mat 1-2
GCA_011420 565.1	*A. alternata*	PN2	33,527,939	Contig	Canu v. 1.6	Oxford Nanopore MiniION	*Brassica juncea*	India	ITS, AA-specific, Mat 1-2
GCA_011420 445.1	*A. alternata*	PN1	33,767,884	Contig	Canu v. 1.6	Oxford Nanopore MiniION	*Brassica juncea*	India	ITS, AA-specific, Mat 1-2
GCA_011420 255.1	*A. alternata*	JS-0527	33,800,049	Scaffold	FALCON v. 0.3.0; SOAPdenovo v. 2.04; SSPACE v. 3.0; SSPACE-LongRead v. 1.1; GapFiller v. 1.10	PacBio Sequel; Illumina HiSeq	*Phragmites australis*	South Korea; Gyeongbuk	ITS, AA-specific, Mat 1-1
GCA_001443 195.1	*A. alternata*	ATCC 34957	33,499,641	Contig	Celera Assembler v 8.2	PacBio	*Sorghum bicolor*	USA: Kansas	ITS, AA-specific, Mat 1-2
GCA_016097 525.1	*A. alternata*	EV-MIL-31	34,964,232	Scaffold	SPAdes v. v3.14.1	Illumina MiSeq; Nanopore GridION	Citrus (Minneola tangelo)	Florida	ITS, AA-specific, Mat 1-2
GCA_014154 925.1	*A. alternata*	B3	33,838,288	Contig	SPAdes v. 3.14.1	Illumina	*Ophiopogon japonicus*	China: Zhejiang	ITS, AA-specific, Mat 1-1

GenBank Accession Number	Alternaria species	Strain	Genome Information				Host	Location	Sequence Obtained
			Total Genome Length	Assembly Level	Assembly Method	Sequencing Technology			
GCA_020449 105.1	*A. alternata*	Hznu325	33,740,545	Contig	SPAdes v. 2021	Illumina	*Chrysanthemum*	China: Tongxiang	ITS, AA-specific, Mat 1-2
GCA_001572 055.1	*A. alternata*	Z7	34,355,870	Contig	CLC NGS Cell v. 8.0.3; HGAP v. 2.0	Illumina HiSeq; PacBio	*Citrus suavissima*	China: Wenzhou	ITS, AA-specific, Mat 1-1
GCA_004154 755.1	*A. alternata*	FERA 1177	35,633,396	Scaffold	SPAdes v. 3.9.0	Illumina MiSeq	*Malus domestica*	United Kingdom	ITS, AA-specific, Mat 1-1
GCA_001696 825.1	*A. alternata*	B2a	33,016,309	Scaffold	CLC NGS Cell v. 7.5.1	Illumina MiSeq	Onion leaf	South Africa: Pretoria-Roodeplaat	ITS, AA-specific, Mat 1-1
GCA_004634 295.1	*A. alternata*	MOD1-FUNGI5	33,434,224	Contig	SPAdes v. 3.8.2	Illumina NextSeq 500	Seedless grapes	USA: MD	ITS, AA-specific, Mat 1-1
AB009451	*A. alternata*	-	-	-	-	-	*Pyrus pyrifolia*	Japan	Mat 1-1
GU735421.1	*A. alternata*	-	-	-	-	-	*Pyrus pyrifolia*	Japan	Mat 1-1
GU735422.1	*A. alternata*	-	-	-	-	-	*Pyrus pyrifolia*	Japan	Mat 1-1
GU735423.1	*A. alternata*	-	-	-	-	-	*Pyrus pyrifolia*	Japan	Mat 1-1
GU735425.1	*A. alternata*	-	-	-	-	-	*Pyrus pyrifolia*	Japan	Mat 1-1
GU735426.1	*A. alternata*	-	-	-	-	-	*Pyrus pyrifolia*	Japan	Mat 1-1
GCA_001642 055.1	*A. alternata*	-	32,990,834	Scaffold 1	-	Illumina	*Oryza sativa*	USA	Mat 1-1
BMP0270	*A. alternata*	-	33,410,000	Scaffold	-	-	*Oryza sativa*	USA	Mat 1-1
ATCC11680	*A. alternata*	-	33,570,000	Scaffold	-	-	*Oryza sativa*	USA	Mat 1-1
ATCC66891	*A. alternata*	-	33,160,000	Scaffold	-	-	*Oryza sativa*	USA	Mat 1-1
MPI-PUGE-AT-0064	*A. alternata*	Altalt1	33,440,000	Scaffold 5	-	Pacbio	*Arabidopsis thaliana*	France	Mat 1-1
GCA_004154 765.1	*A. tenuissima*	FERA 648	33,508,343	Contig	SPAdes v. 3.9.0	Illumina MiSeq	*Pyrus* sp.	United Kingdom	ITS, Mat 1-1
GCA_017589 455.1	*A. tenuissima*	ANJ	33,681,665	Scaffold	SPAdes v. 3.11.1	Illumina HiSeq	Garden soil	India: Durgapur	ITS, Mat 1-2
GCA_004156 01521.1	*A. tenuissima*	FERA 1164	34,724,580	Scaffold	SPAdes v. 3.9.0	Illumina MiSeq	*Malus domestica*	United Kingdom	ITS, Mat 1-2
GCA_004154 735.1	*A. tenuissima*	FERA 24350	33,066,186	Scaffold	SPAdes v. 3.9.0	Illumina MiSeq	*Pyrus pyrifolia*	United Kingdom	ITS, Mat 1-2

GenBank Accession Number	Alternaria species	Strain	Genome Information				Host	Location	Sequence Obtained
			Total Genome Length	Assembly Level	Assembly Method	Sequencing Technology			
GCA_004168 565.1	*A. tenuissima*	FERA 635	36,064,554	Scaffold	SPAdes v. 3.9.0	Illumina MiSeq	*Malus* sp.	United Kingdom	ITS, Mat 1-2
GCA_004154 745.1	*A. tenuissima*	FERA 1082	33,942,725	Scaffold	SPAdes v. 3.9.0	Illumina MiSeq	*Malus* sp.	United Kingdom	Mat 1-2
GCA_004154 845.1	*A. tenuissima*	FERA 743	35,921,821	Scaffold	SPAdes v. 3.9.0	Illumina MiSeq	*Malus* sp.	United Kingdom	Mat 1-2
GCA_004154 835.1	*A. arborescens*	FERA 675	33,943,449	Scaffold	SPAdes v. 3.9.0	Illumina MiSeq	*Malus* sp.	United Kingdom	ITS, Mat 1-2
GCA_004634 205.1	*A. arborescens*	MOD1-FUNGI6	33,822,904	Contig	SPAdes v. 3.8.2	Illumina NextSeq 500	*Malus* sp. (Red apple)	USA:MD	ITS, Mat 1-2
GCA_000256 225.1	*A. arborescens*	EGS 39-128	33,889,384	Contig	Velvet v. 0.7.43	Illumina GA II	*Solanum lycopersicum*	California	ITS, Mat 1-2
GCA_004154 815.1	*A. arborescens*	RGR 97.0016	33,774,152	Scaffold	SPAdes v.3.9.0	Illumina MiSeq	*Malus* sp.	USA (USDA)	ITS, Mat 1-2
GCA_013282 825.1	*A. arborescens*	NRRL 20593	33,589,656	Scaffold	CLC NGS Cell v. September-2018	Illumina MiSeq	*Solanum lycopersicum*	USA: California	ITS
GCA_004155 955.1	*A. arborescens*	RGR 97.0013	33,803,556	Scaffold	SPAdes v. 3.9.0	Illumina MiSeq	*Malus* sp.	USA(USDA)	ITS

Supplementary Table 3: ITS Sequences Representative of *Alternaria* species from Different Host Isolated in Various Geographic Locations

Species	Accession No.	Host	Location	Reference
A. alternata	MG763130	Grapes	Pakistan	S. Ghuffar *et al.,* 2018
A. alternata	MF785102	Grapes	Pakistan	S. Ghuffar *et al.,* 2018.
A. alternata	JX985742	Banana leaf	USA	V. Parkunan *et al.,* 2013
A. alternata	JQ074093	Pigeonpea	India	Mamta, Raju, & Suresh., 2013
A. alternata	LC269968	*Impatiens hawkeri*	Japan	J. Nishikawa & C. Nakashima., 2020
A. alternata	LC440581	*Antirrhinum majus*	Japan	J. Nishikawa & C. Nakashima., 2020
A. alternata	LC269969	*Pelargonium hortorum*	Japan	J. Nishikawa & C. Nakashima., 2020
A. alternata	LC440582	*Primula polyantha*	Japan	J. Nishikawa & C. Nakashima., 2020
A. alternata	LC440583	Tomato	Japan	J. Nishikawa & C. Nakashima., 2020
A. alternata	LC164855	*Vigna radiata*	Japan	J. Nishikawa & C. Nakashima., 2020
A. alternata	KP940475	Onion leaf	South Africa	Bihon *et al.,* 2015
A. alternata	KX118413	Tomato	India: Varanasi	Meena *et al.,* 2016
A. alternata	LM644275- LM644341 (66)	Tomato	Upper Egypt	Gherbawy *et al.,* 2018
A. alternata	JQ340207	Tomato	China: Guizhou, Guiyang	Xie, Tan, & Yu., 2012
A, alternata	MG786770	Tomato	Iran	Ramezani, Taheri, & Mamarabadi., 2019
A, alternata	AF404648	Citrus	South Africa: Mpulamanga	Rang *et al.,* 2002
A, alternata	AF404651	Citrus	South Africa: Mpulamanga	Rang *et al.,* 2002
A, alternata	AF404652	Citrus	South Africa: Western Cape	Rang *et al.,* 2002
A, alternata	AF404655	Citrus	South Africa: Western Cape	Rang *et al.,* 2002
A, alternata	AF404656	Citrus	South Africa: Western Cape	Rang *et al.,* 2002
A. alternata	AF404659	Citrus	South Africa: Western Cape	Rang *et al.,* 2002
A. alternata	AF404664	Citrus	South Africa: Western Cape	Rang *et al.,* 2002
A. alternata	AF397220	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. alternata	AF397222	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. alternata	AF397231	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. alternata	AF404663	Citrus	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	HQ905482	Tomato	Saudi Arabia	Agamy *et al.,* 2013
A. tenuissima	MG786766	Tomato	Iran	Ramezani, Taheri, & Mamarabadi., 2019
A. tenuissima	JX495165	Potato	China: Gansu Province	Zheng & Wu., 2013
A. tenuissima	JX495166	Potato	China: Gansu Province	Zheng & Wu., 2013
A. tenuissima	JX241643	Fuji Apple	China: Shaanxi Province	Gao *et al.,* 2012
A. tenuissima	MK748314	Grapes	China: Shandong Province	Gao *et al.,* 2020
A. tenuissima	AF397224	Apple	South Africa: Western Cape	Rang *et al.,* 2002

Species	Accession No.	Host	Location	Reference
A. tenuissima	AF397233	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397234	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397236	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397237	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397238	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397239	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397241	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397242	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397243	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397244	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF404642	Citrus	South Africa: Mpulamanga	Rang *et al.,* 2002
A. tenuissima	AF404643	Citrus	South Africa: Mpulamanga	Rang *et al.,* 2002
A. tenuissima	AF404645	Citrus	South Africa: Mpulamanga	Rang *et al.,* 2002
A. tenuissima	AF404647	Citrus	South Africa: Mpulamanga	Rang *et al.,* 2002
A. tenuissima	AF397218	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397219	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397223	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397225	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. tenuissima	AF397235	Apple	South Africa: Western Cape	Rang *et al.,* 2002
A. arborescens	JX241641	Fuji Apple	China: Shaanxi Province	Gao *et al.,* 2012
A. arborescens	MG786771	Tomato	Iran	Ramezani, Taheri, & Mamarabadi., 2019