Chapter the most abundant filamentous fungi. for

CHAPTER 5 DISCUSSION Soilis a complex biological system that provides a major function for the livingthings. Fungi in the soil can be used asa bioindicator for biodiversity conservation (Barbosaet al.

, 2016), Fungi are crucial as a component ofsoil microbiota depending on the soil depth and nutrient condition as it can constitutemore in the soil rather than the bacteria. Fungi help in the decomposition ofplant structural polymers such as cellulose, hemicellulose and lignin that canlead to the maintenance of global carboncycle (Chavezet al., 2015).

Fungi such as Aspergillus and Penicillium specieswere the most abundant filamentous fungi. For the purpose of this research, mostof the fungi isolated from the soil at the Block A and behind DK Delta in theFaculty of Applied Sciences, UiTM Shah Alam are among these genera. Inthis research, four fungal cultures were isolated from block A and anotherseven fungal cultures were isolated from behind DK Delta by serial dilutiontechnique. This technique was done to reduce the cell concentration of thefungi systematically so that the number of colonies of the fungi will not be toocrowded in one plate (Sanders, 2012).

The fungi were then sub-cultured and purifiedto reduce any contamination that can cause a major problem in theidentification method. The isolated fungi were identified by a classical way; usingmorphological identification and molecular identification. Even thoughmolecular identification was able to identify rapidly and completely, themorphological identification remains as one of the methods that mostly used foridentification of fungi. This method also helps the researchers to betterunderstanding the fungal growth and

their diversity (Gautam and Bhadauria, 2012). Inthe macromorphological identification, the isolated fungi were grown in differenttypes of media which are Czapek Dox Agar (CDA), Malt Extract Agar (MEA), andPotato Dextrose agar (PDA) so as to differentiate the colony morphology, formand elevation, as well as the reverse and surface colour of the fungi (Kadhim and Al-Hussaini, 2015). Other than that, these different media used for macromorphological can influence thepigmentation and sporulation, vegetative growth and colony morphology dependingto the composition of the media (StanlyPradeep et al., 2013).

Physical andchemical factors can also differentiate the effects in characterisation of thefungi by using these several types of media : the sporulation and mycelialgrowth on artificial media are crucial in biological characteristics (Chaudhuriet al., 2017). Most of the isolated fungi in thisstudy grown on CDA, MEA and PDA showed the various colours colony such as cream, dark green, yellow, and white in the colony morphology and reverse and surfacecolour.

Most of the isolated fungi showed irregular flat and circular flat forms. Micromorphologycharacteristics within a species are more stable and informative as compared tothe colony appearance. The ornamentation of ascospores and their size are themajor informative phenotypic characteristic for species recognition (Chenet al., 2017). In the micromorphologicalcharacteristic, the suspected fungi were observed under 1000x magnificationwith immersion oil after growing them in MEA for about seven days. MEA was usedin this micromorphological study due to the

stripes on MEA that usually are moreconsistent and distinctly rough rather than other media (Samson and Pitt, 2000).

In thismagnification, the structure of the Aspergillusand Penicillium species wereobserved based on their structure of the phialide, vesicle, conidia, conidiophore, hyphae, metulae, and rami. Aspergillus speciesis characterised by having a spore bearing structure known as conidial head, basal foot or hyphae and aseptate. They also have a conidiophore that terminatedinto the vesicle. The vesicles of Aspergillusspecies have one or two layer of same cells and conidial heads that isasexually formed spore produced by the phialides (Nyongesaet al., 2015). Seven samples of suspected Penicillium species were identifiedbased on the basic micromorphological characteristics such as conidia, conidiophore, phialides, rami, metuale, and hyphae that are typical element forPenicillium species. The comparisonin micromorphology of Penicillium species was made based on their branching ofconidiophore, and the shape of the ornamentation of the conidia (Houbrakenet al., 2010).

The260nm/280nm of QuickDrop Spectrophotometer reading of the eleven samples detectedand the positive controls, with most of the samples were less than 1. 8 whichindicates the presence of protein phenol or others. Other than that, only onesample showed a result in the range of 1. 8 to 2. 0 which indicated that thesample has pure DNA (Tan and Yiap, 2009). In addition, there are no sample that showed average reading of more than two which indicatedthe presence of RNA.

The reading was taken repeatedly for three times to obtainthe best results. However, though most of the samples were not in the range of pureDNA, the

samples were used for further analysis since the value of the puritiesis close enough to the range of pure DNA. Althoughthe identification of Aspergillus andPenicillium species was done by usingmorphology (phenotypic characters) which also known as classical method, thismethod was commonly being misleading due to the hybridization, crypticspeciation, and convergent evolution. This method also did not provide anyprecise grouping within the evolutionary framework typically at species level (Rajaet al., 2017). DNA barcoding is a molecularidentification which also known as modern procedure by using ITS region that beingused to align the gene sequence and to identify the suspected Aspergillus and Penicillium species.

Internal transcribed space (ITS) region inidentification of various types of fungi has high success rate which give theclearest define barcode gap between inter and intra-specification variation. DNA barcoding used standardise range between 500 to 800 bp sequences to identifyspecies of fungi by using the primer that can be used to a wide range oftaxonomic groups (Schochet al., 2012). Based on the study, the PCR productof all fungi samples in 1. 4% agarose gel, showed that all bands were in therange of 500 to 700 bp which is in the same range of the Aspergillus and Penicillium speciesand matched with the range size of the primers used (Henryet al.

, 2000; Demirelet al., 2013). ThePCR purification was done after the size of PCR product of the sample is thesame as the expected size for Aspergillusand Penicillium species.

Thepurified PCR also in the same size range with these species. Two samples of purifiedPCR products (F3 and F11) then were sent to the third party for

sequencing sincethey have the highest concentration of DNA and highest DNA purity. Thesequencing result for both samples were shown in Figure 4. 5, 4. 6, 4.

7, 4. 8, 4. 9, 4. 10, 4. 11, and 4. 12. Based on the sequencing result after being cleaned, the size of contig for F3 and F11 sample are 662 bp and 577 bp.

However, bothsamples are not in Aspergillus or Penicillium species, but in the Scolecobasidium sp. and Microsphaeropsis arundinis. Figure 4.

7 and 4. 8 showed the BLAST results for F3. The results showed the description of the alignment of the contig sequences. The identity of both accession number for F3 is KC790476. 1 and KJ942584. 1 were84% indicated that the query's length was 84% identical to the first hit in thenucleotide-to-nucleotide alignment.

The identity was not totally similarbecause the DNA sequences sent by the third-party showed multiple peaksthroughout the sequences due to the sample quality. GenBank Graphics of theaccession number KC790476. 1 was with 36 gaps. The species that was identifiedin the F3 sample is Scolecobasidium sp.

The identity of accession number forF11 is KJ774054. 1 was 100% identical to the first hit in thenucleotide-to-nucleotide alignment. The identity is similar because the DNAsequences that was sent by the third-party showed cleans peaks with low noise peaksthroughout the sequences. This sequence showed that the sample quality for thatpurified PCR is good. The species that

was identified in the F11 sample is Microsphaeropsis arundinis. Even though both species are not Aspergillus and Penicillium species, they shared some of the characteristics of these two fungi. Scolecobasidium sp. is commonly found in soil and water and is a thermophilicdematiaceous fungus (Pundhiret al.

, 2017)and an anamorphic ascomycetes (Renkeret al., 2005). Microsphaeropsis arundinis is adematiaceous mould that inhabits terrestrial plant host and in ubiquitous insoil and fresh water (Pendleet al.

, 2004). Scolecobasidium sp. and Microsphaeropsis arundinis aredeuteromycetes also known as imperfect fungi because they are artificial groupof fungi. Deuteromycetes are an artificial assemble that join ascomycetes andbasidiomycetes asexual stages because there is no sexual reproductive structurethat have been discovered yet (Gams and Seifert, 2001; Lockey and Ledford, 2014).

Aspergillus and Penicillium species belong to the phyla Zygomycota, Ascomycota andDeuteromycetes. These three phyla have different ways to reproduce. Ascomycotahave a mycelium barrier that can reproduce by producing a spore bags, whilezygomycota have the ability to multiply vegetative and produce a spore whereas deuteromycetesonly reproduce vegetatively by conidia (? ukiewicz-Sobczak, 2013). The molecular approach has confirmed deuteromycetes as a lineage to Ascomycetesor Basidiomycetes (Pringle, 2013), so it is not possible if Scolecobasidium sp. and Microsphaeropsis arundinis has somesimilarity with the Aspergillus and Penicillium species. In the antimicrobial activity of the isolated fungitowards

pathogenic bacteria, only one sample showed the inhibition zone and itwas towards the pathogenic bacteria S. epidermidis.

It is Gram-positive bacteria. F4 fungi sample was suspected tobe Penicillium species. This speciescan produce penicillin that can slow down the peptidoglycan of Gram-positivebacteria (Lobanovska and Pilla, 2017). In general, about 70% fungal strains shows theantimicrobial properties against pathogenic bacteria and fungi that slow downthe growth of bacteria was higher than showing the antibacterial properties.

Other than that, most of antimicrobial properties were frequently againstGram-positive bacteria than Gram-negative bacteria. This is due to thecomplexity of the cell wall of the Gram-negative bacteria than theGram-positive bacteria and Gram-negative bacteria can provide additional degreeof protection against antimicrobial properties that aim the cell wall (Zainuddinet al., 2010).

The antimicrobial properties of these fungi can also being influenced by theirhabitat and the lack of significant difference in the size of inhibition which canexist due to the same biochemical pathways used by the bacteria (Waithakaet al., 2017).