Indonesian Journal of Environmental Management and Sustainability
e-ISSN:2598-6279 p-ISSN:2598-6260

IJEMS

Research Paper

# Phylogenetic Analysis Of Endophytic Fungi Isolate from Bellucia pentamera Naudin Based On ITS rDNA

Andika Puspita Dewi[1], Elisa Nurnawati[2], Laila Hanum[2], Hary Widjajanti[2]*

[1]*Department of Biosciences Program. Faculty of Science. Sriwijaya University. Jalan Padang Selasa 524. Palembang. South Sumatra 30139. Indonesia*
[2]*Department of Biology, Faculty of Mathematics & Natural Sciences, Sriwijaya University. Jalan Raya Palembang-Prabumulih km 32, Indralaya, Indonesia*
***Corresponding author e-mail**: harywidjajanti@gmail.com

**Abstract**

Endophytic fungi can produce secondary metabolites. The purpose of this study is to identify and examine the genetic relationship of endophytic fungi isolates with ITS markers. The endophytic fungi isolate DKJ1, DKJ3a, DKJ3c, and DKJ4 were successfully isolated from the Cardia plant (*Bellucia pentamera Naudin*) indicated by *Aspergillus niger* group, *Aspergillus fumigatus* group and *Penicillium sp.* The results of identification and analysis of DNA the sequencing of endophytic fungi DKJ1, DKJ3c, DKJ4, and DKJ3a with the primary pair of ITS shows that the phylogenetic tree is different from the species obtained isolate DKJ1 has a similarity index value of 1,000 with *Aspergillus piperis* CBS 112811 species and 1,000 similarity index values with *Aspergillus luchuensis* KACC 46772 species, DKJ3c has a similarity index value of 1,000 with the species *Aspergillus flavus* var flavus strain ATCC 16833, DKJ4 has a similarity index value of 1,000 with the *Penicillium oxalicum* CBS 219.30 species and has a similarity index value of 1,000 with the *Penicillium oxalicum* strain NRRL 787 species and isolate DKJ3a has a similarity index value of 1,000 with the *Penicillium roflsii* strain NRRL 1078 species. But there are similarities based on Cluster A (*Aspergillus* Group) and Cluster B (*Penicillium* Group) on phylogenetic trees.

**Keywords**
*Endophytic fungi, Inter Transcribed Spacer, Aspergillus, Penicillium*

Received: 5 November 2019, Accepted: 23 November 2019
https://doi.org/10.26554/ijems.2019.3.4.100-106

## 1. INTRODUCTION

Endophytic fungi are capable of producing biological compounds or secondary metabolites that are thought to be a result of coevolution or genetic recombination from host plants to endophytic fungi (Adriani, 2015; Tan and Zou, 2001). Endophytic fungi are microbes that live in plant tissues at certain periods and can live by forming colonies in plant tissues without endangering the host (Kumala, 2015). Endophytic fungi can produce secondary metabolites of endophytic fungi isolated from the host plant (Rahayu et al., 2014). One type of plant that can produce secondary metabolites is Kardia (*Bellucia pentamera Naudin*) and the content of active compounds in the plant are flavonoids, saponin, and tannin (Marisa et al., 2018; Mouratilde et al., 2013).

Isolation of the endophytic fungi of the Kardia plant (*Bellucia pentamera Naudin*) has the potential to produce antibacterial and antioxidant compounds. Endophytic fungi have been identified based on morphological characters. The morphological identification results are endophytic fungi isolates (DKJ1) identified as *Aspergillus niger* group, fungi isolate (DKJ3c) was identified as *Aspergillus fumigatus* group, fungi isolate (DKJ4) and (DKJ3a ) were identified as *Penicillium sp.* The *Aspergillus niger* group has a large number of species with similar morphology, species associated with the *Aspergillus niger* group are *Aspergillus eucalypticola*, *Aspergillus neoniger*, and also famous species namely *Aspergillus carbonarius* and *Aspergillus tubingiensis*, likewise the *Aspergillus fumigatus* group fungi (Samson and Gams, 1984; Samson et al., 2007a). However, this identification has not been able to provide accurate species certainty. This is because some fungi can have the same morphological characteristics, in the form of a colony color, a diameter of the colony, and the color of the media around the colony although the two fungi isolates differed types, namely between the *Aspergillus fumigatus* group and *Penicillium sp.* Therefore, the identification results obtained morphologically still need to be verified by other methods. One method that can be used is to further characterize using molecular data so that kinship or taxonomic status can be determined

(Purnamasari et al., 2013).

This research molecularly identified the endophytic fungi isolates that have been obtained, namely the *Aspergillus niger* group (DKJ1) isolate, *Aspergillus fumigatus* group (DKJ3c), *Penicillium sp.* (DKJ4 and DKJ3a). The identification of the 4 endophytic fungi isolates is still in the group and genus level because the characters obtained are still biased so it cannot provide certainty for identification at the species level. Proper identification to determine species and their kinship is molecular analysis using ribosomal DNA sequence analysis (rDNA) (Mulyatni et al., 2016). Therefore there is no status of this species *Aspergillus niger* group (DKJ1), *Aspergillus fumigatus* group (DKJ3c) dan *Penicillium sp.* (DKJ4 dan DKJ3a), it will be done with ITS rDNA markers. With the hope of the results of this study can find out the species and its taxonomy.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

The used in this study are *Aspergillus niger* group (DKJ1), *Aspergillus fumigatus* group (DKJ3c) and the fungi *Penicillium sp.* (DKJ4 and DKJ3a) from Sriwijaya University Microbiology Laboratory.

### 2.2 Fungi rejuvenation and DNA extraction

DNA extraction reagents used the Tiangen catalog Plant Genomic DNA Kit and after that the DNA samples were stored at -20 °C for long-term storage.

### 2.3 ITS Amplification

The amplification of the ITS area is done using one primer pair. Amplification of the fungi ITS area was carried out using one pair of ITS 1 - ITS 4 primers (White et al., 1990). Primers ITS 1 as forward primers and ITS 4 as reverse primers. Primer used for DNA amplification are ITS 1 5' TCCGTAGGTGAACCTGCGG 3', ITS 4 5'TCCTCCGCTTATTGATATGC 3' (Glass and Donaldson, 1995). Amplification was carried out in a Thermal Cycler 2700 PCR machine with 94 ºC Predenaturation, 94 ºC denaturation, 54 ºC annealing, 72 ºC elongation and 72 ºC post-elongation for ITS amplification with 30x cycles. The tube containing the PCR product was stored at -20 ºC for analysis by electrophoresis.

### 2.4 DNA Sequensing

The sequencing stage is done by sending samples, each of the 20 $\mu$l samples that had been neatly packed and tightly packed in a parafilm-coated box was then sent to 1st BASE Singapore.

### 2.5 Data Analysis

The DNA sequence results are combined using the Bioedit program. Homology of DNA sequence sequences from sequencing results with DNA sequence data that has been reported previously searched using the *Basic Local Alignment Search Tool* (BLAST) site of GenBank site then selected by the similarity of the species seen from the value of the Query cover and its high identification. Phylogenetic tree construction is carried out with the MEGA 7 program.

## 3. RESULTS AND DISCUSSION

### 3.1 DNA isolation of DKJ1, DKJ3a, DKJ3c and DKJ4 fungi

The results of DNA isolation are known through agarose gel electrophoresis. Based on Figure 1 it is known that the DNA of the endophytic fungi of the Kardia plant has been successfully isolated from four day old mycelia. The success of DNA isolation greatly affects the quality of DNA obtained because DNA isolation is the initial stage in molecular identification. DNA band which is the result of isolation can be seen in Figure 1 that there is a DNA band on the electrophoresis gel and the quality of the DNA is carried out electrophoresis on 1% agarose gel.

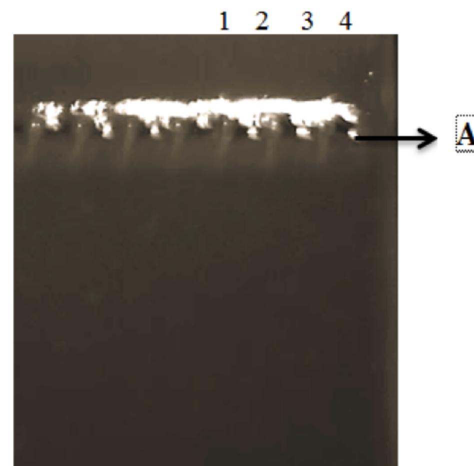


**Figure 1.** DNA electropherogram produced by Isolation of Kardia Plant Endophytic Fungi. Note: A. DNA band, sample isolate (1.DKJ1, 2. DKJ3a, 3. DKJ3c, and 4. DKJ4)

The Kardia plant was isolated but the DNA isolation results obtained were not in the form of a single band, but blotchy or not very clear DNA bands. This is most likely due to the polysaccharides produced by these fungi which are extracted when DNA isolation is carried out (Rahayu et al., 2014; Ethica et al., 2013). The smear shadow pattern under the DNA band shows that DNA does not completely, resulting in the emergence of fragments of different sizes and are retained in agarose gels according to their size which is DNA bands formed. Smear shadow patterns can also indicate the presence of RNA contamination while good isolation results are marked with the resulting clear band and the absence of smear shadow patterns under the DNA band. Nevertheless, this DNA isolate can still be used for PCR amplification (Sauer et al., 1998).

© 2019 The Authors.

**Table 1.** Fragment Sizes of DKJ1, DKJ3, DKJ3c dan DKJ4 ITS region

| No | Sample | 18S (bp) | ITS 1 (bp) | 5,8S (bp) | ITS4 (bp) | 28S (bp) | ITS region (ITS1; 5,8S; ITS4) (bp) | Total (bp) |
|---|---|---|---|---|---|---|---|---|
| 1 | DKJ1 | 20 | 175 | 168 | 164 | 70 | 507 | 597 |
| 2 | DKJ3a | 27 | 173 | 159 | 168 | 63 | 500 | 590 |
| 3 | DKJ3c | 28 | 181 | 158 | 165 | 62 | 504 | 594 |
| 4 | DKJ4 | 33 | 178 | 157 | 168 | 46 | 503 | 582 |

### 3.2 DNA Amplification

The DNA isolates of the Kardia plant endophytic fungi were amplified using an ITS primer pair (ITS1-ITS4) at an annealing temperature of 54° C (White et al., 1990). The PCR products were visualized by agarose gel electrophoresis under UV light to chek the presence of amplified bands. Figure 2 shows a clearly reinforced band (single band) in the range of 500-700 bp resulting from all samples (DKJ1, DKJ3a, DKJ3c and DKJ4) using PCR with specific primers. The success of PCR amplification is influenced by the use of an appropriate primary pair and the right annealing temperature for each fungus (Jamsari and Kasim, 2007). If the annealing temperature is too low then the possibility of non-specific bonds will occur resulting in undesirable products and if the temperature is too high, the primer cannot bind to the DNA template.

DNA sequencing of ITS region using forward primer (F1) and reverse primer (R1) produced nucleotide sequences with size ranged from 582-597 bp which consisted of 18S; ITS 1; 5.8S; ITS 2; and 28S region (Table 1).

According to White et al. (1990) that the primers used in PCR amplification of this DNA isolate using ITS1 and ITS4 pairs are specific sizes for primer combinations in the ITS region and have a size range of 563-602 bp. Research that has been done states by using primer ITS1-ITS4 amplification results obtained along 500-600 bp (Hermosa et al., 2000). Then another study also succeeded in identifying the fungus *Trichoderma sp.* by using ITS1-ITS4 markers the amplification results obtained from 550 to 700 bp (Abd-Elsalam, 2003).

According to White et al. (1990) that the primers used in PCR amplification of this DNA isolate using ITS1 and ITS4 pairs are specific sizes for primer combinations in the ITS region and have a size range of 563-602 bp. Research that has been done states by using primer ITS1-ITS4 amplification results obtained along 500-600 bp (Hermosa et al., 2000). Then another study also succeeded in identifying the fungus Trichoderma sp. by using ITS1-ITS4 markers the amplification results obtained from 550 to 700 bp (Abd-Elsalam, 2003).

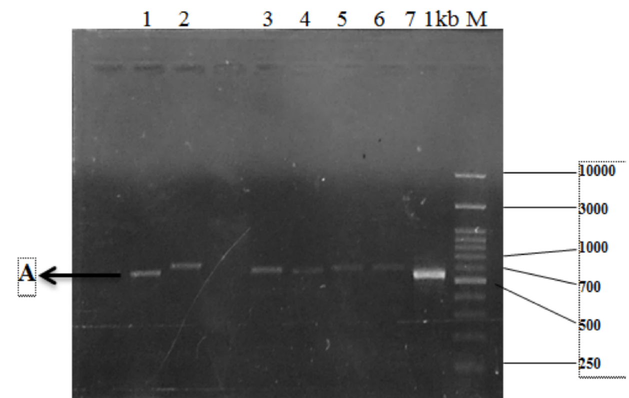


**Figure 2.** Electrophorogram PCR DNA ITS rDNA products. Electrophorogram PCS DNA ITD rDNA products. Note: (A) The size of a single DNA band obtained in the range of 500-700 bp. (M) 1Kb DNA Marker. (1-7) Types of sample isolates (1-2) DKJ1, (3-4) DKJ3a, (5-6) DKJc, (7) DKJ4.

### 3.3 Results of Blast and Phylogenetic Analysis of Bellucia pentamera Naudin's Endophytic Fungi

Based on the analysis of the Basic Local Alignment Search Tool (BLAST) in NCBI, it shows that fungi have similarities to the four isolates of endophytic fungal samples used. The reconstruction was carried out between four sample isolates with six sequences in NCBI to determine the genetic relationship of the sequences of Blast analysis similarity results. The six sequences are *Aspergillus piperis* CBS 112811, *Aspergillus luchuensis* KACC 46772, Aspergillus flavus var flavus strain ATCC 16833, *Penicillium oxalicum* strain CBS 219.30, *Penicillium oxalicum* strain NRRL 787, and *Penicillium rofsii* strain 1078.

The results of the study in Table 2 show that all samples produced similarities in homology values above 90%, namely with *Aspergillus piperis* cbs 112811, *Aspergillus luchuensis* KACC 46772 *Penicillium oxalicum* strain CBS 219.30, *Penicillium oxalicum* strain NRRL 787, dan *Penicillium roflsii* strain NRRL 1078. Percentages above 90% indicate the sequence of nucleotide bases that are homologous to those in GenBank. This is if the degree of homology similarity shows the highest value, the isolates in BLAST can be considered the same species as those on GenBank (Seprianto et al.,

© 2019 The Authors. 

**Table 2.** Homology Search (Searching similarites) on DKJ1, DKJ3c, DKJ4 and DKJ3a based on BLAST

| No | Sample | Result of Similarities from Blast | Identity (%) | Query Coverage |
|---|---|---|---|---|
| 1 | DKJ1 | *Aspergillus piperis* cbs 112811 | 99% | 97% |
| | | *Aspergillus luchuensis* KACC 46772 | 100% | 93% |
| 2 | DKJ3c | *Aspergillus flavus var flavus* strain ATCC 16833 | 99% | 98% |
| 3 | DKJ4 | *Penicillium oxalicum* strain strain CBS 219.30 | 96% | 97% |
| | | *Penicillium oxalicum* strain NRRL 787 | 95% | 96% |
| 4 | DKJ3a | *Penicillium roflsii* strain NRRL 1078. | 93% | 95% |

2018).

Similarity index values of *Aspergillus welwitschiae* CBS 139.54, *Aspergillus foetidus* CBS 121.28, *Aspergillus niger* strain ATCC 16888, *Aspergillus niger*, ITSDKJ1, *Aspergillus piperis* cbs 112811, *Aspergillus luchuensis* KACC 46772, *Aspergillus brasiliensis* strain CBS 101740, *Aspergillus ellipticus* CBS 707.79, *Aspergillus subflavus* CBS 143683, *Aspergillus caelatus* strain CBS 763.97, ITSDKJ3c, *Aspergillus flavus var flavus* strain ATCC 16833, dan *Aspergillus flavus* ATCC 16883, *Penicillium oxalicum* strain CBS 219.30, *Penicillium oxalicum* NRRL 787, ITSDKJ4, *Penicillium pedernalense*, *Penicillium componatum* CBS 140982, *Penicillium roflsii* strain NRRL 1078 and ITSDKJ3a as outgroup were given on Table 3. The highest and lowest sequence similarity index values were 1,000 and 0.957, respectively. Highest sequence similarity index values was found in all samples of DKJ1, DKJ3c, DKJ4 and DKJ3a meanwhile the lowest order sequence similarity index values was found in all Aspergillus and Penicillium species.

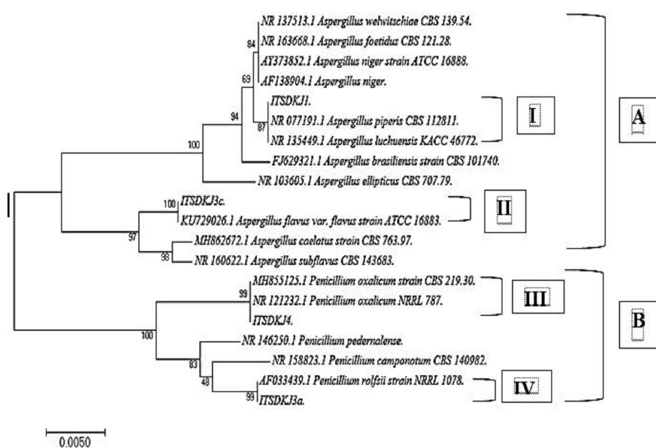


**Figure 3.** Phylogenetic Trees ITS Endophytic Fungi Isolates (DKJ1, DKJ3a, DKJ3c, and DKJ4). Clusters A and B of the Fungi Type are obtained from the Primer ITS. Part I-IV Indicates Species Species that have Similarity to Samples.

The phylogenetic relationship of the endophytic fungi of *Bellucia pentamera* Naudin based on Figure 3 shows that there are two main clusters of phylogenetic endophytic fungi namely Cluster A consisting of the genus *Aspergillus* and ckuster B consisting of the genus *Penicillium.* Based on the grouping pattern that *Aspergillus* and *Penicillium* are monophyletic groups that have high similarities among members. A phylogenetic approach, a group of organisms whose members have a lot of similarities of character is considered to have a very close relationship and estimated descended from a common ancestor (Hidayat and Pancoro, 2016).

The phylogenetic results in cluster A consisted of the *Aspergillus* genus and were divided into two subclasters, namely sub-cluster I and sub-cluster II, there are different results.

Subcluster I isolate DKJ1 with a high coefficient value of 1,000 (Table 3) with *Aspergillus* piperis CBS 112811 species and *Aspergillus luchuensis* KACC 46772 species are the same species. This is supported by the suitability of the coefficient values (Table 3) and 99% similarity rate (Table 2) and morphological characteristics data obtained, the DKJ1 isolate showed the same species as *Aspergillus piperis* CBS 112811 and *Aspergillus luchuensis* KACC 46772. Purnamasari et al. (2013); Samson et al. (2014) reported that sequences from DKJ1, *Aspergillus piperis* CBS 112811 and *Aspergillus luchuensis* KACC 46772 were included in the phylogenetic relationship of members of the *Aspergillus niger* group, then the related species are *Aspergillus welwitschiae* CBS 139.54, *Aspergillus foetidus* CBS 121.28, *Aspergillus niger* strain ATCC 16888, *Aspergillus niger* and *Aspergillus brasiliensis* CBS 101740 strains, *Aspergillus ellipticus* CBS 707.79 included in one clade with subcluster I.

Meanwhile subcluster II consists of DKJ3c and *Aspergillus flavus var flavus* strain ATCC 16833 has a high coefficient value of 1,000. Based on 1,000 high coefficient value (Table 3) and supported by 99% similarity value (Table 2) and morphological data that have been obtained, the DKJ3c isolate can be identified by the same species as the one in Genbank namely *Aspergillus flavus var flavus* strain ATCC 16833. Samson and Gams (1984) Reported that the sequence of *Aspergillus flavus var flavus* strain ATCC 16833 was included in the phylogenetic relationship which

© 2019 The Authors.

**Table 3.** Values of Index of Similarity sequence of DKJ1, DKJ3a, DKJ3c, and DKJ4 ITS rDNA region

| Sample | Similarity | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 1 | | | | | | | | | | | | | | | | | | | | |
| 2 | 0,959 | | | | | | | | | | | | | | | | | | | |
| 3 | 0,959 | 1,000 | | | | | | | | | | | | | | | | | | |
| 4 | 0,957 | 0,997 | 0,997 | | | | | | | | | | | | | | | | | |
| 5 | 0,959 | 0.998 | 0.998 | 0,995 | | | | | | | | | | | | | | | | |
| 6 | **1,000** | 0,959 | 0,959 | 0,957 | 0,959 | | | | | | | | | | | | | | | |
| 7 | 0,963 | 0,973 | 0,973 | 0,972 | 0,973 | 0,959 | | | | | | | | | | | | | | |
| 8 | 0,987 | 0,958 | 0,958 | 0,957 | 0,958 | 0,987 | 0,966 | | | | | | | | | | | | | |
| 9 | 0,963 | 0,973 | 0,973 | 0,972 | 0,973 | 0,963 | **1,000** | 0,966 | | | | | | | | | | | | |
| 10 | 0,987 | 0,958 | 0,958 | 0,957 | 0,987 | 0,987 | 0,966 | **1,000** | 0,966 | | | | | | | | | | | |
| 11 | 0,965 | 0,971 | 0,971 | 0,97 | 0,973 | 0,965 | 0,992 | 0,968 | 0,992 | 0,968 | | | | | | | | | | |
| 12 | 0,959 | 0,998 | 0,998 | 0,995 | **1,000** | 0,959 | 0,973 | 0,958 | 0,973 | 0,995 | 0,973 | | | | | | | | | |
| 13 | 0,960 | 0,99 | 0,99 | 0,99 | 0,99 | 0,960 | 0,974 | 0,958 | 0,974 | 0,958 | 0,972 | 0,99 | | | | | | | | |
| 14 | 0,87 | 0,958 | 0,958 | 0,957 | 0,987 | 0,987 | 0,966 | **1,000** | 0,966 | 1,000 | 0,968 | 0,958 | 0,958 | | | | | | | |
| 15 | 0,958 | 0,998 | 0,998 | 0,995 | **1,000** | 0,959 | 0,973 | 0,958 | 0,973 | 0,958 | 0,973 | 0,100 | 0,99 | 0,958 | | | | | | |
| 16 | 0,959 | 1,000 | 1,000 | 0,997 | 0,998 | 0,959 | 0,973 | 0,958 | 0,973 | 0,958 | 0,971 | 0,998 | 0,99 | 0,958 | 0,998 | | | | | |
| 17 | 0,99 | 0,960 | 0,960 | 0,959 | 0,960 | 0,99 | 0,966 | 0,984 | 0,966 | 0,984 | 0,966 | 0,960 | 0,962 | 0,984 | 0,960 | 0,960 | | | | |
| 18 | 0,991 | 0,959 | 0,959 | 0,958 | 0,957 | 0,991 | 0,962 | 0,981 | 0,962 | 0,981 | 0,961 | 0,957 | 0,961 | 0,981 | 0,957 | 0,959 | 0,992 | | | |
| 19 | 0,963 | 0,972 | 0,972 | 0,973 | 0,972 | 0,963 | 0,992 | 0,967 | 0,992 | 0,967 | 0,997 | 0,97 | 0,971 | 0,967 | 0,972 | 0,972 | 0,966 | 0,961 | | |
| 20 | 0,959 | 1,000 | 1,000 | 0,997 | 0,998 | 0,59 | 0,973 | 0.958 | 0,973 | 0,958 | 0,971 | 0,998 | 0,99 | 0,958 | 0,998 | 1,000 | 0,96 | 0,959 | 0,972 | |

note: 1.*Penicillium roflsii* strain NRRL 1078; 2. *Aspergillus niger*; 3. *Aspergillus niger* strain ATCC 16888; 4. *Aspergillus brasiliensis* strain CBS 101740; 5. ITSDKJ1; 6. ITSDKJ3a; 7. ITSDKJ3c; 8. ITSDKJ4; 9. *Aspergillus flavus var flavus* strain ATCC 16833; 10. *Penicillium oxalicum* strain CBS 219.30; 11. *Aspergillus caelatus* strain CBS 763.97; 12. *Aspergillus piperis* cbs 112811; 13. *Aspergillus ellipticus* CBS 707.79,; 14. *Penicillium oxalicum* NRRL 787; 15. *Aspergillus luchuensis* KACC 46772; 16. *Aspergillus welwitschiae* CBS 139.54; 17. *Penicillium pedernalense*; 18. *Penicillium componatum* CBS 140982; 19. *Aspergillus subflavus* CBS 143683; 20. *Aspergillus foetidus* CBS 121.28

is supported by morphological characteristics, namely A. flavus spore variants that are brownish green to yellowish in color. Related species are *Aspergillus* subflavus CBS 143683 and *Aspergillus caelatus* strain CBS 763.97 included in one clade with subcluster II and has a similarity coefficient value that is close to 0.997. This is because the two isolates have different morphological characteristics.

DKJ1 isolates have characteristics on CDA (*Czapek Dox Agar*) and CYAS (*Czapek Yeast Autolysate*) medium which are white on the edges and black in the middle, maenwhile on MEA (*Malt Extract Agar*) medium, it appears that the colony is solid black and the color is opposite the fungi colony, horned conidia are round, semi-round, brown conidiophores, have a smooth surface and fialids grow above the metula. Whereas DKJ3c Isolate has the characteristic of CDA medium looks greenish-yellow, on CYAS the colony medium looks brownish-yellow. While the MEA medium appears a light green colony and the color opposite is a clear brownish colony. Conidiophores are green in color, smooth surface and in the shape of *Clavate*.

Cluster B from phylogenetic endophytic fungi consisted of the genus *Penicillium*, the fungi isolates identified, namely sub-cluster III (DKJ4) and Sub-cluster IV (DKJ3a) turned out to have different results. Sub Cluster III isolate DKJ4 with a high coefficient value of 1,000 (Table 3) with *Penicillium oxalicum* CBS 219.30 species and *Penicillium oxalicum* strain NRRL 787 species it can be stated that the same species. This is supported by the suitability of the coefficient values (Table 3) and 96% similarity rate (Table 2) and morphological characteristics data obtained, it can be stated that the same species namely *Penicillium oxalicum* CBS 219.30 and Penicillium oxalicum strain NRRL 787.

Meanwhile sub-cluster IV consists of DKJ3a and Penicillium rolfsii strain NRRL 1078 have a high coefficient value of 1,000 Based on high coefficient values (Table 3) and supported by 99% similarity value (Table 2) and morphological characteristics data that have been obtained. This is because the two isolates of DKJ4 and DKJ3a have different morphological characteristics. DKJ4 fungi isolates have grayish-green characteristics on CDA and MEA medium, but their growth is faster in the MEA medium. Conidiophores on branched fungi, have a greenish color, have a smooth surface, and are of the monoverticillate type. Fialid is in the form of *lanceolate* (*acerose*), conidia in the form of semi-round and green. Whereas DKJ3a has the characteristics of white and pink fungi colonies on the CDA medium, grayish-green fungus colonies on the CYAS medium, and MEA, conidiophores are branched and belong to the type of simple *conidiophores* (*monoverticillate*), there is no metula and only phialids in the form of pumpkins (flask-shaped), conidia are green with ellipse shape. This indicates that these fungi isolates belong to the genus *Penicillium*.

Based on phylogenetic data from DNA sequencing from endophytic fungi (DKJ1, DKJ3c, DKJ4, and DKJ3a) Bellucia pentamera Naudin plants that show different results for the species obtained (Figure 3). However, there are sim-

© 2019 The Authors. 

ilarities based on the main cluster divided into two clusters consisting of cluster A (*Aspergillus* group) and cluster B (*Penicillium* group). When viewed from the genetic relationship of the Aspergillus niger group endophytic fungi, that cluster A (Figure 3) is still included in *Aspergillus niger / Aspergillus fumigatus* (Samson et al., 2014, 2007b; Abarca et al., 2004; Kredics et al., 2008; Soares et al., 2012) and cluster B (Figure 3) are still included in *Penicillium* (Samson et al., 2014; Visagie et al., 2014; Samson et al., 2004).

## 4. CONCLUSIONS

DNA extraction of endophytic fungi *Bellucia pentamera Naudin* was successfully amplified using ITS1-ITS4 primer pairs at an annealing temperature of 54 °C and each had a band length of 570 bp. The results of the identification and analysis of DNA sequencing using ITS primers from endophytic fungi (DKJ1, DKJ3c, DKJ4, and DKJ3a) of *Bellucia pentamera Naudin* that show different results in the species obtained, that shows different results in the species obtained, isolate DKJ1 has a similarity index value of 1,000 with Aspergillus piperis CBS 112811 species and 1,000 similarity index values with *Aspergillus luchuensis* KACC 46772 species, DKJ3c has a similarity index value of 1,000 with the species *Aspergillus flavus var flavus* strain ATCC 16833, DKJ4 has a similarity index value of 1,000 with the *Penicillium oxalicum* CBS 219.30 species and has a similarity index value of 1,000 with the *Penicillium oxalicum* strain NRRL 787 species and isolate DKJ3a has a similarity index value of 1,000 with the *Penicillium roflsii* strain NRRL 1078 species.

## 5. ACKNOWLEDGEMENT

We thank to the 2018 Competitive Research Grant for funding this study and the authors thank lecturer Dr. Hary Widjajanti for their critical guidance in this research.

## REFERENCES

Abarca, M. L., F. Accensi, J. Cano, and F. J. Cabañes (2004). Taxonomy and significance of black aspergilli. *Antonie van Leeuwenhoek*, **86**(1); 33–49

Abd-Elsalam, K. A. (2003). Bioinformatic tools and guideline for PCR primer design. *african Journal of biotechnology*, **2**(5); 91–95

Adriani (2015). Aktivitas Antibakterial Fungi Endofit Caulerpa racemosa Terhadap Bakteri Escherichia coli dan Staphylococcus aureus. *Prosiding Seminar Nasional Mikrobiology Kesehatan dan Lingkungan*; 11–15

Ethica, S. N., D. R. Nataningtyas, P. Lestari, I. Istini, E. Semiarti, J. Widada, and T. J. Raharjo (2013). Comparative evaluation of conventional versus rapid methods for amplifiable genomic DNA isolation of cultured Azospirillum sp. JG3. *Indonesian Journal of Chemistry*, **13**(3); 248–253

Glass, N. L. and G. C. Donaldson (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.*, **61**(4); 1323–1330

Hermosa, M., I. Grondona, E. t. Iturriaga, J. Diaz-Minguez, C. Castro, E. Monte, and I. Garcia-Acha (2000). Molecular characterization and identification of biocontrol isolates of Trichoderma spp. *Appl. Environ. Microbiol.*, **66**(5); 1890–1898

Hidayat, T. and A. Pancoro (2016). ULASAN Kajian Filogenetika Molekuler dan Peranannya dalam Menyediakan Informasi Dasar untuk Meningkatkan Kualitas Sumber Genetik Anggrek. *Jurnal AgroBiogen*, **4**(1); 35–40

Jamsari, Y. and M. Kasim (2007). Fenologi perkembangan bunga dan buah spesies Uncaria gambir. *Biodiversitas*, **8**(2); 141–146

Kredics, L., J. Varga, Z. Antal, and R. A. Samson (2008). Black aspergilli in tropical infections. *Reviews in Medical Microbiology*, **19**(3); 65–78

Kumala, S. (2015). Efek antimikroba dari kapang endofit ranting tanaman biduri. *JFIOnline— Print ISSN 1412-1107— e-ISSN 2355-696X*, **7**(2)

Marisa, H., S. Salni, F. Salfamas, and Y. Oktariansyah (2018). STUDI TERHADAP Bellucia pentamera NAUDIN; PERUBAHAN STATUS INVASIF MENJADI BERMANFAAT LARVASIDA. *Prosiding SEMNASTAN*; 44–52

Mouratilde, V., L. A. de Sousa, R. B. de Oliveira, A. M. M. da Silva, H. d. M. Chalkidis, M. N. da Silva, S. Pacheco, R. H. V. Mouratilde, et al. (2013). Inhibition of the principal enzymatic and biological effects of the crude venom of Bothrops atrox by plant extracts. *Journal of Medicinal Plants Research*, **7**(31); 2330–2337

Mulyatni, A. S., A. Priyatmojo, and A. Purwantara (2016). Sekuen Internal Transcribed Spacer (ITS) DNA ribosomal Oncobasidium theobromae dan jamur sekerabat pembanding Internal Transcribed Spacer (ITS) sequences of ribosomal DNA Oncobasidium theobromae and other related fungi as comparison. *E-Journal Menara Perkebunan*, **79**(1)

Purnamasari, M. I., C. Prihatna, A. W. Gunawan, and A. Suwanto (2013). Isolasi dan identifikasi secara molekuler Ganoderma spp. yang berasosiasi dengan penyakit busuk pangkal batang di kelapa sawit. *Jurnal Fitopatologi Indonesia*, **8**(1); 9

Rahayu, F., S. Saryono, and T. T. Nugroho (2014). Isolasi Dna Dan Amplifikasi Pcr Daerah Its Rdna Fungi Endofit Umbi Tanaman Dahlia (Dahlia Variabilis) Lbkurcc69. *Jurnal Online Mahasiswa Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Riau*, **2**(1); 100–106

Samson, R. and W. Gams (1984). The taxonomic situation in the hyphomycete generaPenicillium, Aspergillus and-Fusarium. *Antonie van Leeuwenhoek*, **50**(5-6); 815–824

Samson, R. A., P. Noonim, M. Meijer, J. Houbraken, J. C. Frisvad, and J. Varga (2007a). Diagnostic tools to identify

© 2019 The Authors. 

black aspergilli. *Studies in mycology*, **59**; 129–145

Samson, R. A., P. Noonim, M. Meijer, J. Houbraken, J. C. Frisvad, and J. Varga (2007b). Diagnostic tools to identify black aspergilli. *Studies in mycology*, **59**; 129–145

Samson, R. A., K. A. Seifert, A. F. Kuijpers, J. Houbraken, and J. C. Frisvad (2004). Phylogenetic analysis of Penicillium subgenus Penicillium using partial B-tubulin sequences. *Stud Mycol*, **49**(1); 175–200

Samson, R. A., C. M. Visagie, J. Houbraken, S.-B. Hong, V. Hubka, C. H. Klaassen, G. Perrone, K. A. Seifert, A. Susca, J. B. Tanney, et al. (2014). Phylogeny, identification and nomenclature of the genus Aspergillus. *Studies in mycology*, **78**; 141–173

Sauer, P., M. Muller, and J. Kang (1998). Quantitation of DNA. *Qiagen News*, **2**; 23–26

Seprianto, S., F. Feliatra, and T. T. Nugroho (2018). Isolasi dan Identifikasi Bakteri Probiotik Dari Usus Udang Windu (Penaeus monodon) Berdasarkan Sekuens Gen 16S rDNA. *Biogenesis: Jurnal Ilmiah Biologi*, **5**(2); 83–92

Soares, C., P. Rodrigues, S. W. Peterson, N. Lima, and A. Venancio (2012). Three new species of Aspergillus section Flavi isolated from almonds and maize in Portugal. *Mycologia*, **104**(3); 682–697

Tan, R. X. and W. X. Zou (2001). Endophytes: a rich source of functional metabolites (1987 to 2000). *Natural Product Reports*, **18**(4); 448–459

Visagie, C. M., J. Houbraken, J. C. Frisvad, and A. Et (2014). Identification and nomenclature of the genus Penicillium. *Studies in mycology*, **78**; 343–371

White, T. J., T. Bruns, S. Lee, J. Taylor, et al. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, **18**(1); 315–322

© 2019 The Authors.