The Diversity of Basidiomycota Fungi that Have the Potential as a Source of Nutraceutical to be Developed in the Concept of Integrated Forest Management

Mustika Dewi, I Nyoman Pugeg Aryantha, Mamat Kandar

Abstract: The fungus Basidiomycota found in Indonesia have very high diversity, but have not been explored so far. Development of fungi Basidiomycota is an alternative as a source of natural nutraceuticals, especially beta glucan and lovastatin compounds. This compound can be used in the pharmaceutical and food fields. This study aims to obtain Basidiomycota fungi isolates that have the potential as a nutraceutical source. As the first stage in this research, the activities carried out were exploration, isolation on culture media, and identification of fungi based on genotypic characters. The results showed that the fungi identified based on their genotypic characters were Pleurotusostreatus, Ganodermacf, Resinaceum, Lentinulaedodes, Vanderbyliafraxinea, Auricularia delicate, Pleurotusgiganteus, Auricularia sp. and Tricholomagiganteum. Selected mushrooms that have the potential as a source of nutraceuticals will be further tested to determine the content of lovastatin and beta glucan.

Index Terms: Fungi Diversity; Basidiomycota; Molecular Identification

I. INTRODUCTION

Information about fungus diversity and its use by people is approximately 712,000 species (Mueller et al., 2007). However, in Indonesia only a few types of fungi are used. Production of mushroom represents one of the most commercially important steps towards diversification of agriculture based on microbial technology for large-scale recycling of agro-wastes in an agricultural country (Prakasam et al. 2011). Several types of edible mushrooms have been developed, such as ear mushrooms, straw mushroom , button mushrooms, oyster mushrooms and shiitake mushrooms. The last three fungi, in addition to being edible mushrooms, are also known to have bioactive compounds that function as medicinal or sources of nutraceutical . Cultivation fungi have a lot of diversity, but only a small percentage of them are successfully cultivated. Cultivation fungi that are often found in Indonesia include straw mushrooms, oyster mushrooms, and shiitake mushrooms.

The development of local Basidiomycota mushrooms that are cultivated by utilizing space on the forest floor has not been done mostly in Indonesia. In several countries such as Japan, people have long been cultivating shitake mushrooms by utilizing forest floors. Reported by (Savoie & Largeteau, 2011) that mushrooms from the Basidiomycota group are widely produced in forest areas through the utilization of forest floors as a place to grow these fungi which have economic value quite high by applying the concept of micosilviculture. The concept of micosilviculture is a concept that is applied in the management of integrated forest stands by utilizing forest floor as a place to grow mushrooms.

As the initial step in this study, exploration and identification of fungi obtained from the GunungGeulisSumedang Indonesia forest area were carried out. The fungi that were isolated were then identified molecularly and their diversity based on phylogenetic analysis. The mushroom diversity obtained is expected to be a potential source of nutraceutical and can be developed in forest areas with the concept of integrated forest management.

II. LITERATURE REVIEW

A. Division of Basidiomycota Characteristics

The known number of fungus species up to now is approximately 70,000 of the estimated 1,500,000 species in the world (Blackwell, 2011), in Indonesia there are approximately 200,000 species. Indonesia, which is rich in diversity of plants and animals, also has a very high diversity of mushrooms given its humid environment and tropical temperatures that support fungal growth. The structure of the fungus depends on the type. This fungus has one cell, for example yeast, besides that there are also multicellular fungi and form a fruit body that can reach one meter in size, for example wood mushrooms. Most of the Basidiomycota mushrooms can be consumed, but there are some fungi that can also be deadly. Some members of the Amanita genus contain very deadly

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poisons. Some types of Basidiomycota can also harm plants, for example causing death in field crops. Other basidiomycota division that can be consumed, namely some of the genus Auricularia, Volvariella, and Pleurotus.

The fruiting body of a type of mushroom can be different from other types of mushrooms indicated by differences in cap (pileus), stalk (stipe), and lamella (gills) and cup (volva). There are differences in the size, color, and shape of pileus and the stipe is an important feature in identifying a type of fungus (Blackwell, 2011). Several general characteristics of fungi, namely: mushrooms are organisms that do not has chlorophyll so that its way of life as a parasite or saprofit. Body consists from branching threads called hyphae, a collection of hyphae is called mycelium,the reproduction is asexually and sexually.

The fruiting body of the fungus is composed of fine threads called hyphae. Furthermore, the hypha forms a network called mycelium. This mycelium arranges artificial threads into the fruiting body of the fungus. Hyphae are threadlike structures composed of pipe-shaped walls. The wall covers the plasma membrane and cytoplasm of hyphae. This cytoplasm contains eukaryotic organelles. The hyphae found are mostly limited by transverse walls or septa. Septa has a large pore that is sufficient to pass through the ribosome, mitochondria, and sometimes the nucleus of the cell that flows from the cell to the cell, but there are hyphae that are not septic and are known as senositic hyphae. The structure of senocytic hyphae is known to be produced by division of the cell nucleus many times which is not followed by cytoplasmic division.In general, the fungal hyphae structure has a clamp connection. The presence of a clamp connection in hyphae is a characteristic of fungi belonging to the class of Basidiomycota(Blackwell, 2011).

Basidiomycota are characterized as producing sexual spores called basidiospora. Most members of the basiodiomycota are mushrooms, toadstools and spherical fungi called fleshy mushrooms, which are sexual sporesspread in the air in a different way from other fleshy mushrooms. The structure develops after fusion (fusion) of two haploid hyphae resulting from dikaryotic cell formation. A cell that has both nuclei donated by cells sexually compatible. The diploid cells divide meiosisproduce haploid basidiospores. Basidiospores are released from fungi, spread and germinate into haploid vegetative hyphae.

Basidiomycota mushrooms are one of the foods that are highly nutritious non cholesterol. Some studies show that the average mushroom contains 14-35% protein, calories of 100 kj / 100g and 72% unsaturated fat. Essential amino acids for the body contained in fungi are 9 types, namely lysine, methionine, tryptophan, theonine, valine, leucine, histidine and phenilalanin. Other roles of fungi can be used as antioxidants, anti-inflammatory, immunomodulators, and anti-cancer (Badalyan, 2000; Lindeqiust, Niedermayer, & WD, 2005; Wasser, 2010).

B. Molecular Phylogenetic

Phylogenetics is known as a field related to biological sciences. Phylogenetics provides facilities in the fields of human epidemiology, ecology and biological evolution.Phylogenetics is described as a classification taxonomically from organisms based ontheir evolutionary history, namely their phylogeny and are an integral part of systematic science and have the purpose of determining the phylogeny of organisms based on their characteristics. Further phylogenetics is the center of biological evolution such as the overall shortening of the paradigm of how organisms live and develop in nature (Mount, 2001).

Phylogenetic analysis of amino acid sequences and protein will usually be an important areain sequence analysis. In addition, in phylogeneticscan analyze changes that occur withinthe evolution of different organisms. Based on the analysis,sequences that have closeness can be identifiedby occupying the neighboring branchtree. When the gene family is found inorganism or group of organisms, relationshipsphylogenetics among genes can predictthe possibility that one has a function thatequivalent. Prediction of this function can be tested withgenetic experiment. Phylogenetic analysis tooused to follow changesquickly able to change a species.The analysis of phylogenetic were used three methods, namely (1) Maximum parsimony, (2) Distance, and (3) Maximum likehoood. Those methods generally are applied to construct the evolutionary tree or the best tree for determine sequence variation in group. Every method is usually used for different analysis and data((Mcdonald and Kreitman, 1991; Nielsen and Yang, 1998).

III. METHODOLOGY/MATERIALS

A. Fungal isolates

The fruiting body of the fungus found in the forest area of Geulis Mountain (Sumedang, Indonesia) was isolated by culturing the fruiting body of the fungi in-vitro on PDA (Potato Dextrose Agar) . The stages of isolation carried out are preparation of media, spore culture from the fruiting body of the fungi in the following way: the fruiting body of the fungi is cut off by sterile knife. The sporocarppart was taken and planted in the media of PDA that had been added with Chloramphenicol antibiotics (0.1 g / l). Then placed at room temperature on 28^0C. Furthermore, the growth of mycelium was observed (Alamsjah & Husin, 2010).Data collected was analyzed qualitatively based on characteristics morphology of the mushroom Basidiomycota.

B. DNA extraction, amplification and sequencing

Identification of isolates is carried out by molecular method based on its DNA sequence. Preparation of genomic DNA for the purpose of molecular identification of fungi is carried out as follows: genomic DNA extracted from hyphae from culture results using the modyfied method of CTAB (CationichexadecylTrimethyl Ammonium Bromide). DNA isolation results then carried out amplification with the technique of Polymerase Chain Reaction (PCR) use one primers pair, ITS4

(5'-CCCGCCTGACCTGGGGTCGC-3') as reverse primers and ITS5 (5'TAGAGGAAGGAGAAGTCGTAACAA-3') as forward primers (White et al, 1990). Determination of DNA base sequences is carried out in Macrogen, South Korea. Homology analysis using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The DNA base sequence obtained from the ITS (Internal Transcribed Spacer) area is parallel using the ClustalW computer program (Tamura et al., 2011)

C. Phylogenetic analysis

Phylogenetic analysis was carried out using character DNA, namely the nucleotide sequence from the ITS region. Phylogenetic trees are made using the Mega 5.05 program (Tamura et al., 2011). Kinship analysis using phylogenetic trees can describe the close relationship between fungal species found with comparable fungal species found in the NCBI GenBank based on the results of ITS gene sequence alignment using the ClustalW program (Tamura et al., 2011).

IV. RESULTS AND FINDINGS

In this study mushrooms that grow in the area of Mountain Geulis have been identified based on their genotypic characters and kinship relationships with each other based on the analysis of phylogenetic tree kinship. Discussion of the characteristics and diversity of fungi based on phylogenetic analysis is presented in the following sub-chapters below.

A. Diversity of Basidiomycota Fungi

Characteristics of fungal morphology can be identified through fruiting bodies and colonization of hyphae in the roots (Brundrett, 2004). In this study eight types of fungi can be identified which are included in the class of Agaricomycetes namely Pleurotusostreatus, Ganodermacf, resinaceum, Lentinulaedodes, Vanderbyliafraxinea, Auricularia delicate, Pleurotusgiganteus, Auricularia sp., and Tricholomagiganteum.All fungal isolates can be grown on a PDA culture medium and have almost the same mycelium colony characteristics, which is white color (Fig. 1).

The fruiting body characteristics of oyster mushroom have stems that grow sideways (Latin: pleurotus) and are shaped like oysters (ostreatus) so that oyster mushrooms have binomial names as *Pleurotusostreatus* (Fig. 1A). In the wild nature, oyster mushrooms can be found most of the year in cool mountainous forest areas. The fruit body is seen overlapping on the surface of tree trunks that have decayed or the trunk of a tree that has been cut down because oyster mushrooms are one type of woody fungus. Common media used for growing oyster mushrooms are wood sawdust which is a waste of sawmills. Synytsya et al. (2009) reported that oyster mushrooms are a source of biological glucans and can inhibit the formation or biosynthesis of cholesterol, because it contains statins / lovastatin(Alarcón et al., 2003).

In addition to oyster mushrooms, the mushrooms found in decay wood are ear mushrooms. The mushroom was found to be attached to the bottom of a living tree. This fungus is one group of jelly fungi which belongs to the Basidiomycota class and has a unique jelly texture.In this study two types of ear mushrooms were identified, namelyAuriculariaspdanA.delicate. This fungus is called ear mushroom because the shape of the fruiting body widened like a human ear (ear). The characteristic of this ear fungus is to have a fruiting body that is chewy (like gelatin). The body color of this fruiting body is generally black or blackish brown but some are dark brown. Fruiting body parts from ear mushrooms are shaped like a bowl or sometimes with lobes like ears, having a diameter of 2-15 cm, thin fleshy and chewy (Fig. 1F & 1H).

In forest area was found also fungi of Pleurotusgiganteus (Berk. Karunarathna and K.D. Hyde). The fruiting body of this mushroom has a brown, with brownish gills (Fig. 1G). This fungi has been used as a culinary mushroom and is known to have medicinal properties. Recently, it was reported that P. giganteus contained bioactive compounds that mimic neurite growth factor (NGF) which were accountable for neurite stimulation (Phan CW et al. 2012) possessed liver protection properties (Wong et al., 2012) and were a healthy dietary supplements for brain and cognitive health (Phan, Wong, David, Naidu, & Sabaratnam, 2013).

In this study also found *L. edodes* mushrooms, known as shiitake mushrooms. The fruiting body of this fungus has a brown lid to yellow-brown, with brownish gills and produces rusty spores (Fig. 1D). Surenjava, Zhang, Xua, Zhang, and Zeng (2006)reported the results of his research that the beta-glucans produced by the *L.edodes* fungus had anti-tumor effects. Other mushroom found are *V. fraxinea* which grows on trees near the base of the stem. The fruiting body of the mushroom is purplish red with white edges. The cap of the mushroom is wide and wavy (Fig. 1E). The shape of the fruiting body of the fungus *V. fraxinea* is similar to the fungus *G cf. resinaceum*, but the color of the fruiting body is different. The color of *G cf. resinaceum* fruiting body is blackish brown with white edges (Fig. 1C). The fruiting body is semicircular and does not have a stem but is directly attached to a log. Mushroom caps have a hard texture with a thickness of 1.5 cm. On the upper side of the mushroom cap it has uneven or radial lines and there are small pores on the underside. T.giganteum mushrooms are found near the roots of the tree. The characteristics of the mushroom T. giganteum have a convex to flat cap and brownish color (Fig. 1B). Reported by Prakasam et al. (2011) that T. giganteum contains essential macros, and micronutrients, carbohydrates, proteins, fiber content and also have antioxidant activity.

Fig. 1. Fruiting body and myselium colony of Basidiomycota class: (A) P.ostreatus; (B) T.giganteum; (C) G cf. resinaceum; (D) *L.edodes*; (E) *V.fraxinea*; (F) *A.delicate*; (G) *P.giganteus*; (H) *Auricularia* sp.

A. Diversity of Basidiomycota Based on The Phylogenetic Tree

The phylogenetic tree is a logical approach to show the evolutionary relationships between organisms (Schmidt, 2003). One purpose for

preparing the phylogenetic tree is to establish the proper relationship between organisms as well as estimate the difference from a common ancestor to the offspring (Li, Pearl, & Doss, 1999). To illustrate the relationship between the fungi isolate, we compared their ITS gene sequence to the other fungi species found in the NCBI Gene Bank, using ClustalW program (Tamura et al., 2011). Phylogenetics is described as a taxonomic classification of an organism based on evolutionary historytheir phylogeny and an integral part of systematic science that has a purpose fordetermine the phylogeny of an organism based on its characteristics. Phylogenetic analysis of amino acid and protein sequencesusually it will become an important area in the sequence analysis.The use of phylogenetic species criteria results in recognition of more species than those delimited by morphological characters.

The result showed that the genotypic character of A. delicata was similar to Auriculariasp. with a bootstrap value 100, while P. giganteus was similar to P.ostreatus with a bootstrap value of 100. The genotypic of character of G cf. resinaceum was similar to L. edodes with a bootstrap value 93 (Fig. 2). The bootstraps value is used to test how well the model data set is used. If the bootstrap value is low, the sequence should be excluded from the analysis to obtain a reliable phylogenetic tree (Schmidt, 2003). The genotypic character of V. fraxinea is closely related to the genus Auricularia with a bootstraps value of 64, while T. giganteum is closely related to the genus Pleurotus with a bootstraps value of 75.

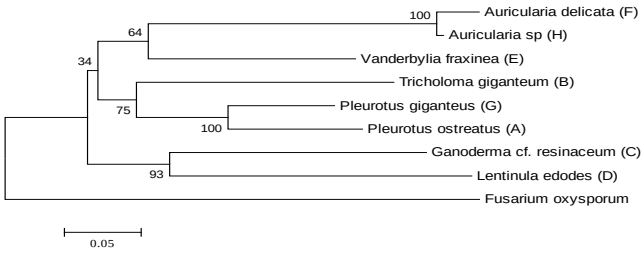

Fig. 2.Dendogram of phylogenetic analysis of Basidiomycota fungi based on ITS sequences using theneighbour joining method with 1000x bootstrap.

V. CONCLUSION

Based on the description described, it can be concluded that the number of Basidiomycota mushrooms found in the area around Mount GeulisSumedang Indonesia is eight species. Based on the results of molecular identification of fungi that were successfully isolated are as follows, namelyPleurotusostreatus, Ganodermacf, resinaceum, Lentinulaedodes, Vanderbyliafraxinea,Auricularia delicate, Pleurotusgiganteus, Auriculariasp., danTricholomagiganteum. The fungi that were isolated were thought to have the potential to become nutraceutical sources.

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