THE COMMUNITY OF SOIL YEASTS IN GUNUNG HALIMUN NATIONAL PARK

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ABSTRACT

Fifty-two isolates were isolated from Gunung Halimun National Park on the basis of morphological and some physiological characteristics. Those isolates were belonged to three groups namely, ascomycetous, basidiomycetous and imperfect yeasts. *Rhodotolum* sp. was only found in Ciptarasa site at 1500 m asl, ascomycetous yeasts was only isolated from deteoretic root in Gunung Botol site, while *Candida* sp. (small globose shaped cells) was only isolated from soil at 1800 m asl of Gunung Botol site. Type of plant species appeared has no effect on yeasts diversity as shown by similar yeasts diversity was observed at rhizosphere soil of three dominating plant *(Schima waallichii, Castanopsis javanica* and *Altingia excelsa)* at Cikaniki study site.

Key words : Ascomycetous yeast, soil yeast.

INTRODUCTION

Yeasts are defined as unicellular fungi reproducing by budding or fission. On the basis of sexual reproduction yeasts can be divided into three groups: ascomycetous, basidiomycetous and imperfect yeasts. Ascomycetous yeasts form ascospore during their sexual stage, basiodimycetous form basidiospore, whereas imperfect yeasts did not perform sexual stage during their life cycle.

Wickerman was the pioneer in identification of yeast in 1951. Since that, the knowledge in yeasts taxonomy has growth rapidly, as shown by the increase of identified species tremendously from 500 species to 700 species (Kurtzman *et al.,* 1998).

Until recently, the study about diversity of yeasts only focus on yeast isolated from traditional fermented foods. However, the study about yeasts diversity and verification characteristic physiological of soil yeast, especially soil in G. Halimun have been not reported yet, in fact some author reported the role of yeasts ecology in soil ecosystem (Lachance, 1990).

Gunung Halimun National Park (GHNP) is one of the most conserve forest ecosystem in tropical area. Since, they endowed with high diversity of plant as well as animal, it is therefore interesting to verify the diversity of soil micro flora especially yeasts. The study of yeasts in that ecosystem is very few though some yeasts have ecological role in natural conservation. They may play role together with other soil microorganism accelerating nutrient cycle. The role of yeasts in geochemical cycling takes second place to that of bacteria. As a decomposer, yeast respires and often performs a fermentative glycolysis, but rather restricted in the nature of the carbon sources they may assimilate. They produce extra cellular enzyme, such as proteinase, celluse, chetinase and amylase (Anna, 1990).

Terrestrial yeasts are most abundant in plant, animal and soil. In term of overall abundance, it would appear that filamentous fungi tend to out number yeast in most soil. As reported by Phaff and Starmer (1987), the existence of yeasts that isolated repeatedly and exclusively from the soil, such as *Lipomyces* species, *Debaromyces* and certain species of *Cryptococcus* suggests that some habitat specificity may be at play. Some genera of yeasts usually found in soil such as *Candida* and *Debaromyces* and the presence of yeasts usually in accordance with bacteria and soil fungi (Cook, 1958).

The objective of our present work is studying the population and diversity of culturable yeasts of soil Gunung Halimun National Park.

MATERIAL AND METHODS

Yeasts isolation

Soil was collected from several study sites located at GHNP. Pre-cultivation was undertaken by shake culture after transferring 10 g of sample into yeast nitrogen base 6.7%, yeast extract 0.1%, malt extract 0.1%, and glucose 20% (pH 6.8). The cultures were then incubated on rotary shaker for three days at 30° C. Isolation was done by plate count methods with three replicates. The isolation medium consisted of yeast extract (3g/l), malt extract (3g/l), bacto peptone (5g/l), glucose (10g/l), agar (20g/l), 0.2% dichloran solution and streptomycin (100U/1), pH 3.7. Cultivation of isolates were performed at 25° C for three days.

Purification

Prior to use, each strain was streaked onto yeast malt extract agar (YM agar) pH 6.5. This followed by incubation at 25°C for 48 hour. Single well-separated colonies of each form are selected and restreaked onto the same media and reincubated. Twice is generally sufficient to obtain pure culture. After 2 days, the colonies were examined using phase contrast microscope for homogeneity. Homogenous strains were then grown in yeast malt extract broth and preserved.

Identification of yeasts

The yeast strains were tested for their characteristics of vegetative reproduction, sexual characteristics, physiological and biochemical characteristics as described by Barnett *et al.* (1990) and Kurtzman *et al.* (1998).

Morphology of vegetative cells

Yeast cells can be globose, subglobose, ellipsoidal, ovoidal, cylindrical, botuliform, bacilliform, apiculate, lunate or triangular. Definition and illustrations of the various possibilities can be found in Ainsworth and Bisby's Dictionary of the fungi (Hawksworth *et al.,* 1995).

Characteristics of sexual reproduction

***Formation of** ascospore*

Sporulation studies were performed using modified YM agar and Kowado agar containing (Potassium acetate 1.5%, Glucose 0.02%, glutathion lOmM, and agar 2%). Strain from 48 hour growing slant were streaked to the above-mentioned sporulation media, then incubated at 25°C for 3 days, and examined for the presence of ascospores. Morphological observation of ascospore was also conducted by scanning electron microscope (SEM).

Physiological and biochemical characteristics

Utilization of carbon compounds

The carbohydrates employed in the assimilation tests included D-glucose, D-galactose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, me-a-D- glucoside, cellobiose, melibiose, lactose, raffinose, melezitose, inulin, starch, erytritol, xylitol, D-mannitol, 2-keto-D-gluconate, D-gluconate, D- glucuronate and citrate. The assimilation media were inoculates with 0.1 ml of a suspension of 2-day-old YM slant culture, and then the tubes were incubated at 25°C. The tests were done on the continuously rotating shaker at 160 rpm, and examined for turbidity during 2 weeks.

Assimilation of nitrogen compounds

The following of nitrogen source are used: potassium nitrate, sodium nitrite, cadaverine dihydrochloride, L-lysine, and glucosamine. The assimilation media were inoculated with 0.1 ml of a suspension of 2-days-old YM slant culture. Growth was observed after 1 week of incubation at 25°C in rotary shaker. When sign of growth is detected, a second tube was inoculated with one loopful from the first to reconfirm the test result.

RESULTS

Table 1. Diversity of yeast in soil of GHNP Ciptarasa site

Soil sample	Number of cell/g soil	Yeasts morphology			
		Candida			
		Small globose	Big globose	Ascomycetous	Basidiomycetous
Soil (600 asl)	22.0 x 10^5		+	+	
Soil (600 asl)	30.2 x1O^3	+	+		
Soil (980 asl)	31.0 x1O^5		+		
Soil (1000 asl)	48.6 x1O^6			+	
Soil (1500 asl)	10.2 x1O^5		+		+
Soil (1500 asl)	73.0 x 10^6	+	+	+	

Abbreviations : asl, above sea level.

The population of yeast in soil is shown in Table 1 indicating that the most abundance of yeast cells is from soil obtained in 1500 asl, about 73.0 x 10^6. Soil sample obtained from 1000 m asl contain 48.6 x 10^6 yeast cells/g soil, follows by soil sample obtained from 980 m asl and 600 m asl namely 31.0 x 10^5, 30.2 x 10^5 respectively.

Table 2, shows the number of yeast cells/g soil sample isolated from Gunung Botol site. Similarly to Ciptarasa site higher altitude appeared to have higher population.

Table 3 shows the diversity of yeasts isolated from various dominating plant in Cikaniki site.

Table 2. Diversity of yeast in soil of GHNP Gunung Botol site

Sample source	Number of cell/g soil	Yeasts morphology		
		Candida		
		Small globose	Big globose	Ascomycetous
Deteriorated stem	24.0 x1O^5			+
Deteriorated root	44.0 x1O^4		+	
Soil (800 asl)	48.0 x 10^5		+	
Soil (1800 asl)	13.3 x 10^6	+	+	

Abbreviations : asl, above sea level.

Table 3. Diversity of yeast in soil of GHNP Cikaniki site

Vegetation	Number of cell/g soil	Yeasts morphology		
		Candida		
		Small globose	Big globose	Ascomycetous
Schima wallichii	18.4 x1O^5	+	+	+
Schima wallichii	37.0 x1O^5		+	
Schima wallichii	22.3 x 10^2			+
Castanopsis javanica	12.0 x 10^5	+		+
Castanopsis javanica	15.2x10^5			+
Castanopsis javanica	10.8 x 10^5	+		
Altingia excelsa	52.0 x 10^3	+	+	+
Altingia excelsa	17.0 x 10^5	+		+
Altingia excelsa	41.9 x 10^5		+	+
Control	19.3 x1O^6	+	+	+

Table 4. Morphological characteristic of yeasts isolated from Ciptarasa site

Isolate No.	Source	Morphological characteristics		Formation of ascospore
		Vegetative cell	Size (um)	
1g	soil 980 asl	globose to oval	6.97-4.85x4.85-1.94	–
2g	soil 980 asl	globose to oval	7.97-4.85x6.85-1.94	-
3g	soil 1000 asl	subglobose	6.88-4.85x4.85-1.94	+
4g	soil 1000 asl	globose	6.97-4.85x4.85-1.94	+
5g	soil 600 asl	globose	3.87-2.91x3.88-2.91	-
6g	soil 1500 asl	globose to subglobose	4.57-3.71x4.88-3.81	-
7g	soil 1500 asl	globose	6.97-4.85x4.85-1.94	+
8g	soil 1500 asl	subglobose to oval	6.87-4.85 x 3.85-2.94	-
9g	soil 1500 asl	globose	7.97-5.85x2.85-1.94	-
10 g	soil 1500 asl	globose	6.66-4.65 x 3.85-2.94	-
H g	soil 600 asl	globose to subglobose	5.76-3.02 x 5.45-3.22	-
12 g	soil 600 asl	globose	6.56-4.02x6.25-4.14	-
13 g	soil 600 asl	subglobose	6.34-4.52x5.91-3.94	-
14 g	soil 600 asl	globose to oval	4.67-2.91x4.88-2.91	-
15 g	soil 600 asl	globose to subglobose	7.89-3.88x4.86-2.91	+
16 g	soil 1500 asl	ellipsoid	11.64-5.82x3.88-1.94	ND

Abbreviations : asl, above sea level; ND, not determined.

Table S. Morphological characteristic of yeasts isolated from Gunung Boto site

Isolate No.	Source	Morphological characteristics		Formation of ascospore
		Vegetative cell	size (um)	
1a	Deteriorated stem	globose to oval	6.23-4.65x4.89-2.13	-
7a	Deteriorated root	globose	6.43-4.60 x 6.85-4.62	+
8a	justright	-	6.96-4.85x4.85-1.94	
10a	soil 800 asl	globose to oval	7.12-3.88x4.96-3.68	-
11a	soil 1800 asl	globose to oval	7.19-5.16x4.28-2.18	-
12a	soil 1800 asl	globose	6.97-4.85x4.85-1.94	-
13 a	soil 1800 asl	subglobose to oval	4.57-3.78 x 4.88-3.68	-
14 a	soil 1800 asl	globose to oval	6.87-4.85 x 3.85-2.94	-
15a	soil 1800 asl	globose to oval	6.76-4.65 x 3.85-2.94	-
16 a	soil 1800 asl	globose to subglobose	5.76-3.02 x 5.45-3.22	-

Soil samples were collected from Ciptarasa site in various altitudes from 600 m asl until 1500 m asl. From six samples were isolated a total of 16 isolates were listed in Table 4.

From Gunung botol study site, eleven isolates were isolated (Table 5) which characterized by small globose cells (2.66-1.62X 2.86-1.86) urn and big globose cells (7.97-5.85X 2.85-1.94) um in size.

As shows in Table 1, it is clear that there was a variability in number of yeast cells, however, the reason for variability in it's ecosystem has not yet clearly verified, but amount of available organic substrate especially low molecular weigh and soluble organic fraction, humidity, nutrient status and rate of organic matter hydrolysis may affect greatly on the cell division and growth. As reported by Anna (1990), that yeast require significant amount of an organic source of carbon and energy of relatively small molecular weight.

significant amount of an organic source of carbon and energy of relatively small molecular weight.

As noted by Takashima *et al.* (1995), that unique morphology of yeast that form hyphae enabling it to penetrate and liquefy semisolid substrate or spread over smooth, inert surface. That morphological and physiological characteristic of yeasts could be the explanation of their abundance at the high altitude.

Soil pH seems has no great effect on the yeast population density since yeasts usually grow over a broad range of pH values (3.5-7), allowing them in particular to colonize materials that have already been the site of fermentative activities by bacteria (Kurtzman *et al.,* 1998).

Higher dominating of yeasts also companies high population of yeasts at higher altitude. Six isolates belonged to three group of yeast namely ascomycetous, basidiomycetous and imperfect yeasts isolated at 1500 m asl. Interestingly, basidiomycetous belonged to genus of *Rhodotorula* sp, only found in 1500 m asl indicating that higher altitude has higher diversity of yeasts.

To study diversity of yeast originated from decomposed organic materials at Gunung Botol research site, various samples were collected from deteriorated root, decomposed stem and two type of soil obtained from 800 asl and 1800 asl. As shows in Table 2, high population of yeasts could be due to soil contained of various organic substances, decayed woods, decomposed litter and low molecular substances of end product of organic material decomposition.

Niche of yeast mostly be rich in simple organic carbon, liquid or very high in moisture, acidic or occasionally alkaline and nutritionally complex, such condition are met by plant tissue undergoing various form of decay as well as exudates of roots, leaves or flowers.

As shows in Table 3, yeast population of soil rhizosphere of three dominating plants shows no consistent result, implying that vegetation has no special influence on species diversity of culturable yeast. This especially clearly observed in imperfect yeasts *{Candida)* and ascomycetous group. Phaff and Starmer (1987), observed, the existence of yeasts that are isolated repeatedly and exclusively from the soil, such as *Lipomyces* species, *Debaromyces* and certain species of *Cryptococcus* suggest that some habitat specificity might be at play. Our present result have shown that yeasts ecology in soil of GHNP is further necessary to be verified especially on physiological characteristic of isolated strains.

From table 4, it shows clearly that on the basis of morphological characteristic and formation of ascospore during their sexual stage the sixteen isolates can be divided into three groups: ascomycetous, basidiomycetous and imperfect yeasts.

Ascomycetous yeasts

One isolate from soil in 600 asl, two isolates from soil in 980 asl and one isolate from 1500 asl belong to this group (Table 4). The cells of those isolates are globose to oval 6.88-4.85X 4.85-1.94 urn. Those isolates formed one to four round shape ascospores in the ascus during their sexual stage on Gorodkowa and Kowado medium. Urease and DDB (diazonium blue) color reaction were negative. These morphological and physiological characters indicate that those isolates were belonged to the ascomycetous group. However, those isolates still needed further study to be correctly identified at the species level.

Basidiomycetous **yeasts**

On the basis of positive reaction for urease and DBB test, one isolate belongs to this group (Table 4). Cells were ellipsoid 11.64-5.82 X 3.88-1.94 urn, and have a pink colony. Based on these characteristics this isolate belongs to the genus of *Rhodotorula* sp. It is interesting to note that basidiomycetous group could be only isolated from

Bartha (1993) observed that the distribution of yeasts in soil is affected by several ecological factors such as organic material composition, microclimate, and soil humidity.

Table 6. Morphological characteristic of yeasts isolated from *Cikaniki* site

Isolate No.	Source	Morphological characteristics		Formation ofascospore
		Vegetative cell	size (um)	
1	*Schima wallichii*	globose	2.66-1.62x2.86-1.86	-
2	*Schima wallichii*	globose to oval	3.88-2.84 x 3.86-2.94	-
3	*Schima wallichii*	Subglobose	2.56-1.62x2.86-1.86	-
4	*Schima wallichii*	Globose to oval	6.97-4.85x4.85-1.94	-
5	*Schima wallichii*	Globose	7.86-3.91x7.88-2.91	-
6	*Schima wallichii*	globose to subglobose	5.82-3.71x4.88-3.81	-
9	*Schima wallichii*	Globose	6.97-4.85x4.85-1.94	-
10	*Schima wallichii*	subglobose to oval	6.87-4.85 x 3.85-2.94	-
11	*Schima wallichii*	globose	7.97-5.85x2.85-1.94	-
14	*Schima wallichii*	Globose	6.66-4.65 x 3.85-2.94	+
15	*Schima wallichii*	globose to subglobose	5.76-3.02 x 5.45-3.22	+
16	*Schima wallichii*	Globose	6.56-4.02x6.25-4.14	+
e	*Castanopsis javanica*	Globose to subglobose	6.34-4.52 x 5.91-3.94	+
f	*Castanopsis javanica*	globose to oval	3.66-1.91x3.88-1.91	-
g	*Castanopsis javanica*	globose to subglobose	2.89-1.26x2.86-1.24	-
h	*Castanopsis javanica*	globose	6.34-4.52 x 5.91-3.94	+
b	*Altingia excelsa*	Globose to oval	6.86-4.62 x 6.88-4.68	+
k	*Altingia excelsa*	Globose	6.66-4.65 x 3.85-2.94	-
i	*Altingia excelsa*	globose to subglobose	3.89-2.26 x 3.86-2.24	-
d	control	globose	6.34-4.52x5.91-3.94	+
j	control	globose	7.64-4.42 x 6.78-4.62	+
3'	control	Globose to oval	6.86-4.62x6.88-4.68	-
4'	control	globose to subglobose	3.89-2.26 x 3.86-2.24	-
5'	control	globose to subglobose	3.68-2.18x3.75-2.94	-
6'	control	Globose to oval	6.86-4.62 x 6.88-4.68	-

Imperfect yeasts

Eleven isolates were belonged to this group (Table 4). These isolates characterized as follows: produce no ascopore during their sexual stage, gave negative reaction to urease and DBB test. These special characteristics indicated that all isolates in this group belong to the genus of *Candida* sp. On the basis of morphological characteristics, eleven isolates could be divided into two groups namely small globose cells (3.87-2.91X3.88-2.91 urn), and big globose cells (7.97-5.85X2.85-1.94 um) in size.

Candida sp could be isolated from various altitudes implying that they were dominant species at Cipta Rasa. Goto *et al.* (1987) also observed that Candida sp is frequently isolated from soil ecosystem.

To study yeast diversity of GHNP, various sample were collected from Gunung Botol study site include decomposed root and decomposed stem.

On the basis of the data shows in Table 5, eleven isolates could be divided into two groups namely: ascomycetous yeasts (1 isolate, characterized by globose shape ascospore, gave negative reaction for urease and DBB test. Cells size around 6.43-4.60x6.85-4.62 um, isolated only from deteoritic); and imperfect yeasts isolate number 13a characterized by small globose 4.57-3.78x4.88-3.68 um, and nine isolates with big

number 13a characterized by small globose 4.57-3.78x4.88-3.68 µm, and nine isolates with big globose 7.19-5.16x4.28-2.18 µrn. It is interesting to point out that the small globose cells could be only isolated from soil in 1800 asl. However the other size cells could be found in deteoretic stem, soil in 800 asl and soil 1800 asl.

The localities of small globose yeast in 1800 m asl, and Ascomycetous yeast from deteriorated root are physiologically and ecologically intriguing to be further verified. Attention should be focused on its substrate absorption affinity, micronutrient requirements, and growth characteristic and isolation technique for the slow growing yeasts.

Interaction between plant and yeasts

To study the interaction between yeast and dominating plant, a study plot was established at 1100 m asl located in Cikaniki study site (Table 6), at which dominated by *Schima wallichii, Castanopsis javanica* and *Altingia excelsa.* Twenty-five isolates were obtained and morphologically they were divided into two groups namely ascomycetous, and imperfect yeasts.

Ascomycetous yeasts

Eight isolates were belonged to this group have ability to produce a round shape ascospore during their sexual stage (Table 6). The spore could be clearly visualized by special medium and staining procedure follows Schaefer-Fulton's modification. Cells were globose to oval (6.86-4.62 X 6.88-4.68 µm) in size and gave negative reaction for urease and DBB test.

Ascomycetous yeasts appeared to be dominant species in Cikaniki study site, as indicated by Table 6 which shown that this group were present at each plant rhizosphere tested and also in control (no plant). It could be barely concluded that there was no specific interaction between the diversity of yeasts and dominating plant or the type of yeasts is not depending on the type of plant.

Imperfect yeasts

Nineteen isolates were belong to this group, and identified as *Candida* sp (Table 6). There were two type of cells morphology, one small globose and the other was big globose. Small globose cells (2.56-1.62X 2.86-1.86 µm), however big globose (7.97-5.85X 2.85-1.94 um) in size. Similarly to Ascomycetous yeasts, imperfect yeasts were also common. This again verified that yeasts diversity was not affected by plant species.

GHNP endowed with a high physiologically and morphologically heterogeneous yeasts, to better understand its presence and its ecological role, intensive physiological and taxonomic study should be further conducted.

CONCLUSION

1. There were three group of yeasts found in Gunung Halimun National park, namely ascomycetous, basidiomycetous and imperfect yeasts.
2. Altitude appeared to affect species diversity.
3. High altitude has higher species diversity.
4. There was no consistent result on the effect of vegetation on species diversity.

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