IDENTIFICATION OF YEASTS ISOLATED FROM GUNUNG HALIMUN NATIONAL PARK

[Identifikasi Khamir pada Taman Nasional Gunung Halimun]

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ABSTRAK

Dua puluh sembilan isolat khamir diisolasi dari tanah Taman Nasional Gunung Halimun. Sumber isolat berasal dari batang pohon lapuk, akar lapuk yang diambil dari Gunung Botol, Cikaniki, dan Cipta Rasa untuk dipelajari aspek taksonominya. Berdasarkan atas karakter morfologi dan fisiologi, isolat-isolat tersebut digolongkan kepada kelompok ascomyceteous, basidiomyceteous dan imperfect khamir. Selanjutnya ketiga golongan tersebut dimasukkan ke dalam sepuluh kelompok (Kelompok 1 sampai X). Dari 29 isolat tersebut, 7 isolat dimasukkan ke dalam kelompok I diindentifikasi sebagai *Debaryomyces hansenii,* 6 dalam kelompok II sebagai *Candida* sp, 2 dalam kelompok III sebagai *Pichia membranafaciens,* 5 isolat dalam kelompok IV sebagai *Candida galacta,* 1 dalam kelompok V sebagai *Candida sake,* 4 dalam kelompok VI sebagai *Cryptococcus humicolus,* 1 dalam kelompok VII sebagai *Rhodotorula minuta,* 1 dalam kelompok VIII sebagai *Candida* sp, 1 dalam kelompok *Candida* sp, dan 1 dalam kelompok X dalam *Candida* sp. Macam sampel tampaknya tidak berpengaruh kepada keragaman jenis khamir seperti ditunjukkan oleh jenis yang sama diisolasi dari berbagai jenis sampel. Dari banyaknya jenis khamir yang diisolasi menunjukkan bahwa keragaman jenis khamir di Taman Nasional Gunung Halimun tergolong tinggi.

Key words: khamir ascomycetous/ ascomycetous yeasts; khamir basidiomycetous/ basidiomycetous yeasts; khamir imperfect/ imperfect yeasts; Taman Nasional Gunung Halimun/ Gunung Halimun National Park.

INTRODUCTION

The taxonomic studies on yeast have been mainly carried out on yeasts isolated from Indonesian traditional fermented food such as *tape, tempe* or *oncom.* However, the study about yeasts diversity and verification characteristic physiological of yeasts isolated from soil in the tropical rain forest especially in Gunung Halimun National (GHNP) Park have not been reported. Few reports have been made on study of yeasts diversity isolated from plant in Japan (Hamamoto *et al.,* 1998; Gibas *et al,* 1998). Buzzini *et al.* (2000) conducted a research on biodiversity of yeasts isolated from Brazillian rain forest. In fact some authors reported the role of yeast ecology in soil ecosystem (Lanchance, 1990).

Gunung Halimun National Park is one of the most conservedd forest ecosystem in tropical area, since they endowed high diversity of plants as well as animals; it is therefore interesting to verify the diversity of soil microflora especially yeasts. The study of yeasts in that ecosystem is very few though some yeast have ecological role in natural conservation. They may play role together with other soil microorganism accelerating nutrient cycle. The role of yeast 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, cellulase, chetinase and amylase (Anna, 1990).

This paper deals with the isolation **and** identification of culturable yeasts from sources collected in Gunung Halimun National Park.

MATERIAL AND METHODS

Yeast strains and cultivation.

Twenty-nine strains isolated from soil in GHNP and 4 reference strains were used in this study. Isolation sources of yeast strains are presented in Table 1. The strain were maintained on Yeast Malt Extract Agar (pH 6.8) containing

1.0% D-glucose, 0.3% yeast extract, 0.3% malt extract, 0.5% bacto peptone (Difco Laboratories, Detroit, USA) and 1.5% agar (DIFCO.Lab). Strains were cultivated at 25 ° C for 3 days.

Isolation of yeast strains.

Yeast strains were isolated from soil collected from different altitudes, deteriorated stems and deteriorated roots. Enrichment media was used for the isolation of yeast. The enriched medium was composed of 20% D-glucose, 6.7% yeast nitrogen base, 0.1% yeast extract and 0.1% malt extract (pH 6.8). Precultivation was undertaken by shake culture after transferring 10 g of sample into enrichment medium. The cultures were then incubated on rotary shaker for three days at25°C.

Purification.

Prior to use, each strain was streaked onto YM agar pH 6.5. This followed by incubation for 48 hours. 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 two days, the colonies were examined using microscope for homogeneity.

Microorganisms used as reference strains were *Saccharomyces cerevisiae* LIPIMC, *Debaryomyces hansenii* JCM 5912^T, *Rhodotorula minuta* TUA 0960^T and *Candida catenulata* JCM 1604^T.

Phenotypic characterization.

Cells form and size were determined for cell grown on yeast malt extract medium. Otherwise stated, isolates were cultivated at 25 °C. Definition and illustration of the various cell form possibilities can be found in Ainsworth and Bisby's Dictionary of the fungi (Hawksworth *et al.,* 1995). Sporulation studies were performed using Kawado medium containing 0.02% D-glucose, 1.5% potassium acetate, lOmM glutathion and 2% agar. Strain from 48 hours growing slant were streaked to the above mention medium, then incubated at 25 °C for 3 days.and examined for the presence of ascospore. Morphological observation of ascospore was also conducted by scanning electron microscope (SEM).

Utilization of carbon compounds.

The assimilation of carbon compunds was carried out by the method Kurtzman *et al.,* (1998). The carbon assimilation medium containing 0.67% yeast nitrogen base and 5% of carbon sources. 35 of carbon sources was employed in this study. The assimilation media were inoculated with 0.1 ml of suspension of 2 days old YM slant culture, and then the tube were incubated 25 * C. The test were done on the continuously rotary shaker at 160 rpm and examined for turbidity during 2 weeks.

Utilization of nitrogen compounds.

The following nitrogen assimilation medium containing 11.7% yeast carbon base and five nitrogen sources are used in this study. The assimilation media were inoculated 0.1 ml of suspension of 2 days old YM slant culture, and then the tube were incubated 25 * C. The test were done on the continuously rotary shaker at 160 rpm and examined for turbidity during 2 weeks.

The urease production, and formation of starch like compound were tested using solid media, and the results were recorded after 8 days incubation. Diazonium Blue B (DBB) test was carried out according to the method by Kurtzman *et al,* 1998. The test for extracellular deoxyribonuclease (Dnase) activity was carried out following to Kurtzman *et al.,* 1998.

RESULTS

A total of 29 isolates were selected as yeasts (Table 1). One isolate was isolated from rhizosphere of *Castanopsis javanica,* one isolate from rhizosphere of *Schima wallichii,* two isolates from forest floor and seven isolates from rhizosphere of *Schima wallichii* in Cikaniki site. Samples were taken from soil collected from Cipta Rasa site, two isolates were isolated from 1000 m asl, three isolates from 600 m asl and three isolates

from 1500 m asl. One isolate was isolated from deteriorated root, five isolates from 1800 m asl soil and five isolates from deteriorated stem. All samples were collected from Gunung Botol site.

Morphological and physiological characteristics

The morphological characteristics of 29 isolates was shown in Table 1. The cells of all isolates were varied from oval, globose, subglobose and ellipsoid. The cells measured ranging from 3.88 to 4.90um by 11.64 to 4.57 u.m by (Fig. I).

On YM agar after 2 weeks at 25 *C, the streak culture of the most isolates were non pigmented. Only one isolate produce pink pigment when grown in YM agar (strain no. 16.S.1500, Table 1). On the basis of ascospore formation nine isolates formed one to four round shape ascospore in the ascus during their sexual stage on Gorodkowa Kurtzman *et al.,* (1998) and Kowado 1998) media ((Fig. 2). Two isolates formed one to three hate-shape ascospore during their sexual stage in the same above media. The ascospore formation observation for seventeen isolates show that those isolates did not produce ascospore during their sexual stage.

Phenotypic characterization of the isolates studied are shown in Table 2. On the basis of Dnase, ureasc and DBB test, twenty four isolates gave negative reaction for those test, however five isolates gave positive reaction (strain no. 3.s. 1000, 4.S.1000, 10.s.l500, ll.s.600 and 16.S.1500 - Table 1). Based on the results show above, out of 29 isolates is suggested to be divided into ten groups.

Group 1 contained seven isolates (14.r.Sc, 15.r.Cj, 3.ds, 4.ds, 5.ds, fi.ds and 7.ds). All isolates in this group did not have ability to ferment glucose and galactose. Most of the isolates were negative for cellobiose, lactose, inulin, strach, D-arabinose, L-arabinose, methanol and ethanol. Except for one isolate (7.ds) grew well in the presence of D-xylose and L-arabinose as a sole carbon sources. Most of isolates grew well in the medium contain of galactose, sucrose, maltose, mellibiose, raffinose, melezitose, L-rhamnose. glycerol, erythritol, dulcitol, D-mannitol, succinate, citrate, D-glucuronic and xylitol. All isolates were negative for nitrate and nitrite as a sole nitrogen sources but grew well in the presence of cadaverine and L-lysine.

Six isolates were belong to group II (4.r.Sc, 5.r.Sc, 6.r.Sc.,9.r.Sc.,10.r.Sc.,and ll.r.Sc). They have phenotypic characterization as follow: gas production begins after 3-4 days and a full tube of gas develop after 6-10 days during the fermentation of glucose, but all isolates did not produce gas during fermentation of galactose. All isolates were negative for most of the carbon sources tested, however they grew well in the presence of galactose, sucrose, maltose, mellibiose, melezitose, D-xylose, L-arabinose, D-arabinose and erythritol. For nitrogen sources nitrate and cadaverine are not assimilated. The six isolates gave positive reaction for nitrite and L-lysine.

Group III contained two isolates (2.k. and 3.k.). The two isolates did not grow in the presence of most carbon sources tested, except for glycerol and lactate gave a positive reaction. Negative reaction was observed in the presence of sodium nitrate and sodium nitrite, but positive reaction found for cadaverine and L-lysine.

Group IV contained five isolates (12.S.600., 12.S.1800., 14.S.1800., 15.S.1800 and 16.S.1800.). Assilimation of most of the carbon sources tested were negative except for D-mannitol and succinate are assimilated. The same features also found for assimilation of nitrogen sources, all isolates did not assimilate sodium nitrate, sodium nitrite, cadaverine and L-lysine.

Group V only contained one isolate (11.s.l 800). The isolate was positive for fermentation of glucose and galactose. The negative results observed in the presence of cellobiose, lactate, mellibiose, raffinose, inulin, L-arabinose, D-arabinose, L-rhamnose, glycerol, erytritol, dulcitol, D-mannitol and D-glucuronic. Assimilation of galactose, sucrose, maltose,

melezitosc. D-xylosc, D-mannitol, 2-ketogluconic acid, DL- lactate. succinate, citrat and xylitol were positive. The isolates did not grow in the presence of sodium nitrate and nitrite, hence it is grew well in the medium contain cadaverine and L-lysine as a sole nitrogen sources.

Four isolates belongs to group VI (3.s. 1000., 4.S.1000., 10.s.l 500., ll.s.600.). They gave negative reaction for fermentation of glucose and galactose. The members of this group grew well in the medium containing most of the carbon sources tested but they did not grow at the expense of inulin, strach, D-arabinose, DL- lactate, succinate, D-glucuronic and ethanol. Furthermore, all isolate grew well in the medium with sodium nitrate, cadaverine and L-lysine use as a nitrogen sources. In contrast, they did not grow in sodium nitrite.

The member of group VII (16.S.1500) did not produce gas during the fermentation of glucose and galactose. The isolate tended to show scanty growth in the medium containing galactose, sucrose, cellibiose, mellezitose, D-xylose, L-arabinose, glycerol, 2-ketogluconic, DL-Iactate, succinate and xylitol. Negative results were found for the other carbon sources tested. No isolate grew in all nitrogen sources tested.

The same features with group V was also foundin this group, which contained only one isolate (R.I). Most of the carbon sources tested did not assimilate by this isolate, however positive reaction were observed in sucrose, maltose, DL-Iactate, succinate. methanol and ethanol as a carbon sources. Assimilation of sodium nitrate and sodium nitrite were negative, in contrast the member of group grew well at cadaverine and L-lysine as a nitrogen sources.

Group IX contained one isolate (9.S.1500). It also can ferment glucose and galactose during fermentation stage. The isolate can use most of the carbon sources tested. The negative results were found when it grew in inulin, starch, DL-lactic acid, succinic acid and citric acid as a carbon sources. It gave negative reaction for sodium nitrate, but gave positive reaction for another nitrogen sources tested.

Only one isolate belonged to group X (I3.S.600). It can produce gas during glucose and galactose fermentation. Ii has ability to use most of the carbon sources tested except for lactose, inulin. starch, D-arabinose, L-rhamnose, and glycerol. For nitrogen sources, the isolate gave negative reaction for sodium nitrate, however it grew very well in the present of sodium nitrite, L-lysine and glucosaminc as sole of nitrogen sources.

Table 1. Morphological characteristics of yeast strains isolated from Gunung Halimun National Park

Strain No.	Sources	Size (|j,m)	Ascospore formation	Group
14.r.Sc.	*Schima wallichii*	6.66-4.65 X 3.85-2.94	+	I
lS.r.Cj.	*Castanopsis javanica*	5.87-3.54X4.07-3.01	+	I
3.ds.	*Deteriorated stem*	6.57-3.86X3.98-2.98	+	I
4.ds.	*Deteriorated stem*	6.67-4.55 X 3.79-3.22	+	I
5.ds.	*Deteriorated stem*	6.58-3.90X3.80-3.40	+	I
6.ds.	*Deteriorated stem*	6.60-4.46 X 4.04-3.08	+	I
7.ds.	*Deteriorated stem*	6.96-4.85 X 4.85-2.94	+	I
9.r.Sc.	*Schima wallichii*	5.97-3.85X3.85-1.94	-	II
4.r.Sc.	*Schima wallichii*	5.76-3.03X5.45-3.22	-	II
5.r.Sc.	*Schima wallichii*	5.89-4.04X3.90-2.08	-	II
6.r.Sc.	*Schima wallichii*	5.78-3.90X3.98-3.00	-	II
lO.r.Sc.	*Schima wallichii*	5.80-4.02 X 4.24-3.20	-	II
ll.r.Sc.	*Schima wallichii*	5.96-3.80X4.02-2.98	-	II
2.k.	Control	6.34-4.52 X 5.91-3.94	+	III
3.k.	Control	6.86-4.62 X 6.07-4.05	+	III
16.S.1800	Soil 1800 asl	6.76-3.89 X 6.45-3.22	-	IV
12.S.1800	Soil 1800 asl	6.97-4.85X4.85-1.94	-	IV
12.S.600	Soil 600 asl	6.56-4.02X6.25-4.14	-	IV
14.S.1800	Soil 1800 asl	6.87-4.85 X 3.85-2.94	-	IV
15.S.1800	Soil 1800 asl	6.76-4.65 X 3.85-2.94	-	IV
11.s.l800	Soill800asl	7.19-5.16X4.28-2.18	-	V
3. s. 1000	Soil 1000 asl	6.88-4.85X4.85-1.94	-	VI
4. s. 1000	Soil 1000 asl	6.97-4.85X4.85-1.94	-	VI
10.S.1500	Soil 1500 asl	6.66-4.65 X 3.85-2.94	-	VI
ll.s.600	Soil 600 asl	6.56-3.98X4.05-2.87	-	VI
16.S.1500	Soil 1500 asl	11.64-5.82X3.88-1.94	-	VII
R.I.	Deteriorated root	6.43-4.60 X 6.85-4.62	-	VIII
13.S.600	Soil 600 asl	4.67-2.91X4.88-2.91	-	IX
9.S.1500	Soil 1500 asl	4.57-3.71 X 4.90-3.81	-	X

Abbreviation : asl, above sea level.

Table 2. Physiological characteristics of yeast isolated from Gunung Halimun National Park

Isolate number	Ascospore	Diase	DBB urease	F-1 glucose	F-2 galactose	C-1 Glucose	2 Galactose	:-3 sucrose	-4 Maltose	i cellobiose	-6 lactose	mellibiose	3 raffinose	melezitose	-10 inulin	-11 starch	2 D-xylose	C L-arabinose	C-14 D-arabinose	C-15 L-rhamnose
14.r.Sc.	+	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+
15.r.Cj.	+	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+
3.ds.	+	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+
4.ds.	+	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+
5.ds.	+	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+
6.ds.	+	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+
7.ds.	+	-	-	-	-	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+
9.r.Sc.	-	-	-	+	-	+	+	+	+	-	-	+	-	+	-	-	+	+	+	-
4.r.Sc.	-	-	-	+	-	+	+	+	+	-	-	+	-	+	-	-	+	+	+	-
5.r.Sc.	-	-	-	+	-	+	+	+	+	-	-	+	-	+	-	-	+	+	+	-
6.r.Sc.	-	-	-	+	-	+	+	+	+	-	-	+	-	+	-	-	+	+	+	-
lO.r.Sc.	-	-	-	+	-	+	+	+	+	-	-	+	-	+	-	-	+	+	+	-
ll.r.Sc.	-	-	-	+	-	+	+	+	+	-	-	+	-	+	-	-	+	+	+	-
2.k.	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.k.	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16.S.1800	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12.S.1800	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12.S.600	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I4.S.1800	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15.S.1800	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11.s.l 800	-	-	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-
3.S.1000	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+
4.S.1000	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+
I O.s.l 500	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+
ll.s.600	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-
16.S.1500	ND	+	+	-	-	+	+	+	-	+	-	-	-	+	-	-	+	+	-	-
R.I.	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
9.S.1500	-	-	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+
13.S.600	-	-	-	+	+	+	+	+	+	+	-	+	-	+	-	-	+	+	-	-
Saccharomyces cerevisiae	+	-	-	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-
Debaromyces hansenii iCM 5912	+	-	-	-	+	+	-	+	+	+	-	-	+	-	+	+	+	+	+	+
Rhodotonda minuta TUA 0960 Y	-	+	+	-	+	+	+	+	-	-	-	-	-	+	-	-	+	+	-	-
Candida catenulata JCM 1604	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2 continued

Isolate number	C-16 glycerol	C-17 Erythritol	C-18 Dulcitol	C-19 D-mannitol	C-20 2-ketogluconic	C-21 D-L- lactic acid	C-22 Succinic acid	C-23 Citric acid	C-24 D-glucuronic	C-25 xylitol	C-26 methanol	C-27 ethanol	N-1 Nitrate	N-2 Nitrite	N-3 cadaverine	N-4 L-lysine	N-5 glucosamine	T 25 C	T 34 C	T 37 C
14.r.Sc.	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	-
15.r.Cj.	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	-
3.ds.	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	-
4.ds.	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	-
5.ds.	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	-
6.ds.	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	-
7.ds.	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-
9.r.Sc.	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
4.r.Sc.	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
5.r.Sc.	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
6.r.Sc.	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
10.r.Sc.	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
11.r.Sc.	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
2.k.	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-
3.k.	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-
16.s.1800	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-
12.s.1800	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-
12.s.600	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-
14.s.1800	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-
15.s.1800	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-
11.s.1800	+	-	-	+	+	+	+	+	-	+	-	-	-	-	+	+	-	+	-	-
3.s.1000	+	+	+	+	+	-	+	+	+	-	+	-	+	-	+	+	+	+	+	-
4.s.1000	+	+	+	+	+	-	+	+	+	-	+	-	+	-	+	+	+	+	+	-
10.s.1500	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	+	+	+	+	-
11.s.600	+	+	+	+	+	-	+	-	+	-	-	-	+	-	+	+	+	+	+	-
16.s.1500	+	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-	+	+	-	-
R.1.	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	+	-	-
9.s.1500	+	+	+	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	-	-
13.s.600	-	+	+	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	-	-
S. cerevisiae	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-
D. hansenii JCM 5912	+	+	-	+	+	-	+	-	+	+	-	-	-	-	-	-	-	+	+	-
R. minuta TUA 0960 Y	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-
C. catenulata JCM 1604	+	-	-	+	-	+	+	+	-	-	-	-	-	-	+	+	-	+	+	+

Abbreviation: JCM - Japan Collection for Microorganisms; TUA - Tokyo University of Agriculture.

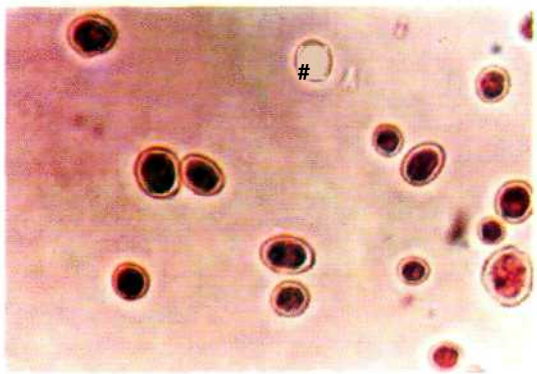

Fig.1. Vegetative cells of *Debaryomyces hansenii* No. 14.r.Sc. in YM medium after 3 days at 25°C. Bar 5 urn.

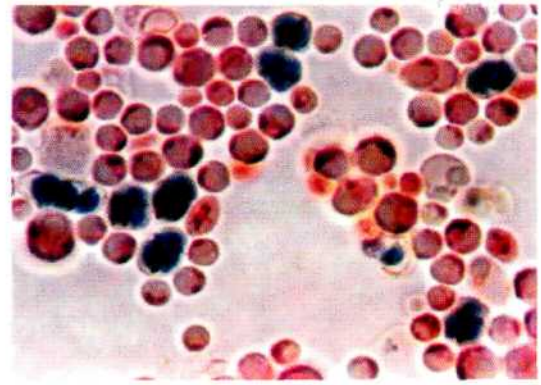

Fig.2. *Pichia membranafaciens* No. 2k, asci with ascospore, after one month on Kawado agar at 25°C. Bar 5 urn.

DISSCUSSION

All yeasts, isolated from various sources in GHNP were classified into ten groups on the basis of morphology and physiological characteristics. Seven isolates which belongs to group I were assigned to the ascomyetous yeasts well accorded to morphological characteristic formed an ascospore in the ascus during their life cycle and some physiological characteristics Dnase, urease and DBB tests were negative.

From the above results, we emphasize that ascospore formation observation was found to be useful for differentiating ascomycetous from other imperfect yeast. We pointed out that the medium Kawado (1998) was more sensitive for the growth of ascomycetous yeast. We also found that scanning electron microscope is useful and important tool to identified yeasts on the basis of ascospore formation. By using this methods, we can observed" the shape of ascospore clearly and more reliable.

As mentioned by Kurtzman *et ai,* (1998) the shape of ascospore is one of important key to identified yeasts into genus level. The member of this group examined in the. present study had round shape ascospore (Fig. 2), lacked the ability of glucose and galactose fermentation. These characteristics are those of the species *Debaryomyces hansenii.* Therefore these isolates were identified as *D. hansenii.* This results support the previous study of Cook (1958) that some genera of yeasts usually found in soil such as *Debaryomyces* and the presence of yeasts usually in accordance with bacteria and fungi.

All the yeast isolates in group **II** were similar in morphology and shared similar physiology characteristics. They examined in the present study did not produce ascospore during their sexual stage, had ability to ferment glucose, and demonstrated a negative Dnase, DBB colour reaction and weak urease activity. These characteristics are those of the group imperfect yeast. On the basis of phenotypic characteristics can only identified at genus level as *Candida* sp. Chemotaxonomic study is needed to identify them up to species level.

Two isolates in the group III belongs to ascomycetous yeasts. However, they had hat-shaped ascospore that found for differentiation of a member in this group from other isolates in group I. From the above results shown in Table 2, we emphasize that those isolates were closely related to *Pichia membranafaciens,* and identified as this species. In this study we isolated those species from forest floor. This results in accordance with Spencer *et al.,* (1997) pointed out that terrestrial yeasts are most abundance in plant and soil, however in term of overall abundance, it would

appear that filamentous fungi tend to out number yeasts in most soil.

All the yeast isolates belong to this group (IV) were similar in morphology and shared similar physiological characteristics features. No exclusive phenotypic characteristic was found for differentiation of each member in this group. The four members in this group isolated from soil at 1H00 asl were classified in genus *Candida,* because they do not form ascospore, gave negative reaction for Dnase DBB, urease. Therefore they identified at genus level as *Candida* sp.

Candida is the biggest genus of yeasts and comprises about one-third of all yeast species, and is still considered to be the most heterogeneous genus (Suzuki *et al.,* 1998). All isolates were isolated from soil 1800 asl. According to previous studies reported by Cook (1958), Nakase *el al.* (1998) and Kurtzman *et al.,* (2000) *Candida* spp is one yeast genera that usually found in soil.

In accordance with this results, Anna (1990) pointed out that yeasts requires significant amount of an organic sources of carbon and energy of relatively small molecular weight. As also noted by Takashima *el al.* (1995) 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 characters of yeast could be the explanation of their abundance at the high altitude.

One isolate from soil in 1800 asl was subjected to identification test. According to the results explained above the isolate was closely related to *Candida glabrata.* However, this isolate fcriVeili Vo vmtiVcVi *Avt' vdavriCviaAwM". characteristics by C. *glabrata* by one or two keys.

On the basis of physiological test, 1 l.s.1800 has ability to assimilate soluble starch as a sole carbon source. The assumsance for that phenomenon was it has ability to convert polysaccharide into low molecular weight of glucose. In 1998, Spencer *el al.,* reported that niche of yeast usually 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 root, leaves or flower.

Four isolates in this group isolated from soil in different altitude however, they were similar in morphological and physiological characteristics. They had exclusive physiological characteristics as found for differentiation of ascomyceotus yeast from basidiomycetous one. Those characters include the positive reaction for Dnase, DBB, and Urease test.

On the basis of identified test was conducted in this study, they close related to *Ctyptococcus Iwmicolus,* and identified as this species. The above results supported the previous study by Phaff and Starner (1987), observed that *Ciypiococcus* was isolated repeatedly and exclusively from soil, suggests that some habitat specificity might be play for supporting the live of this genus.

When the physiological characteristics, the Dnase, urease and DBB test were combined, one isolate of this group were identified as *Rhodotontla* strains. It is emphasized in this study that 16.s. 1500 was found to be closely related to *Rhodotorula minuta.* Out of 29 isoJates were used in this study, only the member of this group produce water soluble-pink pigment, as a one specific pigment produced by this group. Kurtzman *et al.* (1998) reported that this species is prevalent and has a widespread occurrence on a variety of substrates.

The remaining three group (VIII, IX and X) belonged to anamorphic ascomycetous yeast well accorded to morphological and physiological characteristics i.e. did not produce ascospore during their sexual stage, Dnase, urease and DBB negative. However they differ in its physiological characteristics especially in the assimilation of melezitose, D-arabinose, L-rhamnose, glycerol, DL-lactic acid and succinic acid. Thus the three isolates belonging to separate group in the same genus *Candida* sp.

CONCLUSION

Gunung Halimun as last submantone forest in Java has quite high yeasts diversity. Taxonomically they arc divided into ten groups of which group I had largest species members and identified as *Deharyomyces lictnsenii.*

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