DIVERSITY AND ECOLOGICAL PERSPECTIVE OF SOIL YEAST IN GUNUNG HALIMUN NATIONAL PARK

[Khamir Tanah Taman Nasional Gunung Halimun: Keragaman dan Perspektif]

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ABSTRAK

Taman Nasional Gunung Halimun merupakan hutan hujan tropika yang berada di pulau Jawa. Penelitian ini ditujukan untuk mengetahui populasi dari khamir tanah pada berbagai ketinggian tempat (600 m, 1000 m, dan 1500 m) di atas permukaan laut, dan selanjutnya mempelajari peran ekologi dari khamir, dilakukan dengan mengisolasi dan menganalisa karakter fisiologi terutama dalam mendekomposisi selulosa dan hidrolisa fosfat. Khamir yang diisolasi dari tanah dan daerah perakaran dimurnikan dan ditumbuhkan pada media yang mengandung carboxymethylcellulose (CMC) sebagai sumber karbon utama, dan juga ditumbuhkan pada media pivoskaya untuk mengetahui kemampuan pelarutan $Ca_3(PO4)_2$. Populasi khamir pada daerah yang atas sedikit lebih tinggi . Sebanyak 23 isolat khamir yang termasuk dalam marga *Debaryomyces, Pichia, Rhodotorula,* dan *Candida* diisolasi dari tanah. 15 isolat mempunyai kemampuan menghidrolisa selulosa dan 9 isolat mampu melarutkan $Ca_3(PO4)_2$. Khamir ditemukan di tanah dan daerah perakaran dan memegang peran penting dalam degradasi senyawa organik dan mineralisasi fosfat di dalam tanah.

Key word: Khamir tanah, *Debaryomyces, Pichia, Rhodotorula* dan *Candida,* keanekaragaman, perspektif ekologi.

INTRODUCTION

The Gunung Halimun National Park (GHNP) is one of the most conserved forest ecosystems in tropical area with a high diversity of flora and fauna. Though there is incomplete scientific justification, but it is believe that high species richness of flora and fauna is also relevant to that of high microbial diversity

Recently there is growing interest on studying the ecological perspective of yeast in soil ecosystem. It is known that terrestrial yeast is mos* abundance in plant, animal and soil, but our understanding on its significant ecological influence in its habitat is limited. Wickerman was the pioneer in identification of yeast in 1951; Alexander (1961) reported a wide variety of yeast encountered in soil include *Candida, Crytococcus, Debaryomyces, Hansenula, Lipomyces, Pichia, Pullularia, Rhodotorula, Saccharomyces, Schizoblastoporion, Torula, Torulasspora, Torulopsis, Trichosporon* and *Zygosaccharomyces.* Since that the knowledge in yeast taxonomy has growth rapidly, as shown by the increase of identified species tremendously from 500 species to 700 species (Kurtzman, 1998). Since the yeast grow readily at pH 4.0, no difficulty is encountered in the enumeration of yeast, and most of the bacteria and fungi could not grow well at low pH. Enumeration of yeast in the presence of the large number of filamentous fungi common to soil, on the other hand is difficult because the later proliferate more readily and tend to overgrow the former. However a medium at pH 3.8 to 4 contains 0.35 % sodium propionate suppresses both the bacteria and mold so that yeast count can be made. The abundance of these organisms varies greatly with the location *understudy. Yeast* play role together with other soil microorganism accelerating nutrient cycle. As a decomposer yeast often perform as a fermentative glycolyses, but rather restricted in the nature of the carbon source they may assimilate. They produce extra cellular enzyme such as proteinase, cellulase, chitinase and amylase (Anna, 1990). But not many intensive studies conducted to verify which species yeast is ecologically essential and play significant role on

ecosystem sustainability. Recently scanning electron microscope (SEM) have been successfully helping taxonomist to look into deeper morphological characteristic of yeast, and here with SEM is used to observe *in-situ* morphological characteristic of soil yeast.

MATERIAL AND METHODS

Yeasts isolation

Soil, and rhizosphere soil was collected from several study sites located at GHNP (600 m, 1000 m, and 1500 m) asl. 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/L), pH 3.7. Cultivation of isolates was 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.

Observation of **soil** yeast by SEM

About 0.5 g soil sample were added with 1 ml cold ethanol for about 1 h, and remove the alcohol and replace it with 2.5 % glutaraldehyde solution for several hours or more at 4°C. Immerse the material into 2 % tannic acid solution for 6 hours at 4 C. Sample washed with buffer for 15 minutes at 4°C, and repeat it 4 times. Immerse into 1 % OsO_4 solution for 3 h at 4°C, and water washed for 10 minutes, and this procedure was repeated 3 times. Gradient dehydration with 50 %, 75 %, 87,5 % ethanol at 4°C, each step was conducted for 20 minutes."Final dehydration with alcohol absolute for 20 minutes at room temperature. The sample was glued on stab, and coated with gold platinum. Observation was conducted using SEM at 5000 x magnification.

Cellulofytic ability.

All strains were grown on 1 % CMC containing media (Enari, 1983) and the media was added with 0.1 % congored. Clearing zone formation around growing colony was an indication of cellulolytic activity (Joson and Coronel, 1986). After 5-day incubation cellulolytic ability was determined, the ratio the are of clearing zone to colony was calculated.

Phosphate dissolving ability

Strain were grown in Pivoskaya medium contained 5 g l^{-1} $Ca_3(PO_4)_2$1''' , 10 g l^{-1} glucose, 0.2 g l^{-1} NaCl, 0.2 g l^{-1} KC1, 0.0025 g T^{-1} $MnSO_4.H_2O$, 0.1 g l^{-1} $MgSO_4$ $7H_2O$, 0.0025 g l^{-1} $FeSO_4$ $7H_2O$, 0.5 g l^{-1} yeast extract. Formation of clear zone around growing colony indicate Ca_3 $(PO_4)_2$ dissolution.

RESULTS

Yeast in soil is dominated by several genera include *Debaryomyces, Pichia, Rhodotorula* and *Candida,* which belonged to group imperfect yeasts *{Candida* small globose, *Candida* big globose), Ascomycetous yeasts *(Debaromyces, Pichia)* and Basidiomycetes yeasts *(Rhodotorula).* Highest population was observed in 1500 m asl. Altitude appear to affect yeast population and its diversity of ascomyceteous, basidiomyceteus and imperfect yeast. Higher population at higher altitude could be due to high acidity of soil at higher altitude (Rahmansyah *et al.,* 2002), and yeast is preferable at lower pH, while growth of other organism is suppressed.

Yeast population and dominating genera

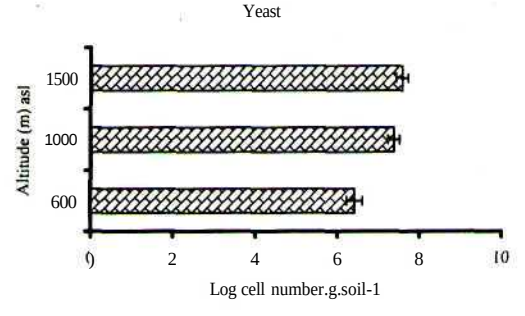

Figure 1. Yeast population in soil

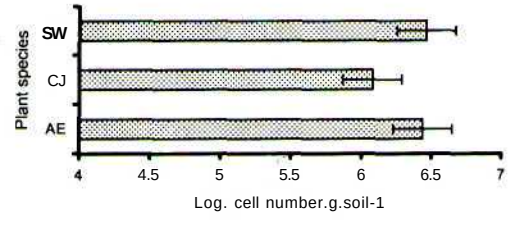

Figure 2. Population of yeast in rhizosphere of *Altingia excellsa* (AE), *Schima wallichii* (SW) and *Castanopsisjavanica* (CJ).

Out of 23 isolates testes 19 isolates were able to hydrolyze CMC. Not much report has quantified the ability of yeast to hydrolyze cellulose. Complete degradation of cellulose were executed by cellulase complex enzyme system include exo-P-1,4 glucanase, endo—P-1,4 glucanase, P-glucosidase (Enari, 1983). Hydrolyzes of CMC indicate the activity of endo—p-1,4 glucanase (Enari, 1983). In soil yeast may collaborate and co-exist among the soil microflora component, and its presence may significantly contribute to bioconversion of organic material in soil. The complexity of cellulose molecule of plant origin may affect cellulose degradation rate. Yeast together with fungi and bacteria may produce different cellulose enzyme system and the presence of that organism in soil accelerates decomposition of organic material in soil. The activity of those complex enzyme is significantly affected by the nature of soil ecosystem include species composition, soil humidity, temperature, the presence inhibitor/stimulator, pH temperature, aeration status and redox potential state of existing environment.

Phosphatase activity.

Most of the strain tested solubilize $Ca_3(PO_4)_2$ (Table 2) indicating that they play on mineralization of phosphate in soil. Soil is a source of nutrient and micro and macro element for microorganism and plant growth. Phosphorous is essential element required by microorganism for nucleotide synthesis and for plant photosynthesis (Tisdale *et al,* 1985). Most soil P is unavailable since it is bound to macro element such as $Ca_3(PO_4)2$, rock phosphate and to organic substances. Most soil consists of organic and inorganic phosphorous. The quantity of inorganic phosphorous in soil mineral is higher than that of organic phosphorous i.e., about 25-90 % of the total soil-P. However in organic soil the quantity of organic-P is in the range of 50-90% (Cosgrove, 1967). The major constituent of organic-P is phytin and inositol. Phytic acid is representing about 60 % of the total phosphorous in soil and mostly accumulated in soil since it is less soluble (Anderson, 1988). The upper layer soil contained more organic-P than subsequent layer.

Soil-P species is mostly pH dependent, and ionic phosphorous is mostly in the form of $H_2PO_4^-$, HPO_4^{2-}, and PO_4^- , it is formed from ionization of H_3PO_4". Ion H_2PO_4" is easier absorbs by plant. The optimal pH for phosphorous ionization is near neutral value and slightly acidic. The presence of soil ionic macroelement such as Fe, Al, Ca and Mn, amount and decomposition stage of organic substances affect solubility of inorganic phosphorous (Brady, 1974).

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Complete degradation of cellulose were executed by cellulase complex enzyme system inclu V exo-pi,4 glucanase, endo-pl,4 glucanase, p-glucosidase (Enari, 1983). Hydrolyzes of CMC indicate the activity of endo-P 1,4 glucanase. In soil yeast may collaborate and coexist among the soil microflora component, and its presence may significantly contribute to biocoversion of organic material in soil. The complexity of cellulose molecule of plant origin may affect cellulose degradation rate. Yeast together with fungi and bacteria ay produce different cellulose enzyme system and the presence of that organism in soil accelerates decomposition of organic material in

soil (Cook, 1958; Willet, 1989; Ruiz et a1, 2000). Activity of those complex enzyme is significantly affected by the nature of soil ecosystem include species composition, soil humidity, temperature, the presence inhibitor/stimulator, pH, aeration status and redox potential state of existing environment (Rao, 1982; Kimnis, 1989).

CONCLUSION

A wide diversity of soil was encountered in GHNP, they belonged *Debaryomyc.es, Pichia, Rhodotorula* and *Candida,* and 19 strain were cellulolytic yeast and 13 isolates were able to solubilize phosphate implying that they have significant role in element mineralization and conversion of organic substances

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