CELLULOLYTIC YEAST ISOLATED FROM SOIL GUNUNG HALIMUN NATIONAL PARK

[Khamir Selulotik yang Diisolasi dari Tanah Taman Nasional Gunung Halimun]

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ABSTRAK

Beberapa khamir tanah yang lermasuk dalam marga *Debaryomyces* dan *Candida* diisolasi dari tanah Taman Nasional Gunung Halimun. Kemampuan selulotiknya diuji dcngan menumbuhkannya pada media yang mengandung carboxymethyl cellulose (CMC) sebagai sumber karbon utama. Pertumbuhan biomassa dan pH diikuti selama kultivasi. Pengamh penambahan glukosa terhadap aktivitas selulolitik juga dipelajari. Dari 23 isolat yang diuji, 19 termasuk khamir selulolitik, dan 1 isolat yang mempunyai kemampuan tertinggi dipelajari karakterisktik ezymatik sclulasenya. Penambahan glukosa menstimulasi pertumbuhan sel dan menyebabkan kebutuhan sel akan glukosa bertambah, dan sclanjutnya memacu sintesa ensim. Dari isolat-isolat yang diuji, isolat *Debaryomyces* S-6 mempunyai aktifitas paling tinggi yaitu sebesar 12 UI pada 96 jam inkubasi. Selama waktu kultivasi pH meningkat dari 6,5 menjadi 7. Adanya khamir yang menghidrolisa selulosa mengindikasikan khamir memegang peranan penting dalam transformasi bahan organik dalam tanah.

Key words: Khamir selulolik / Cellulolytic yeast, khamir tanah/ soil yeast, *Debaryomyces*, Candida, Taman Nasional Gunung Halimun/ Gunung Halimun National Park.

INTRODUCTION

The 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.

Cellulolytic microorganism have been gained popularity (Abe *et al.*, 1979; Blackall *et ah*, 1985; Bélaich *et* «/., 1997), and much microbial inventory executed to collect yeast that have ability to hydrolyze polymeric substances from natural environment. It is known that terrestrial yeast is most abundance in plant, animal and soil (Nakase *et a!.*, 1994), but our understanding on its significant ecological influence in its habitat is limited. Wickerman was the pioneer in identification of yeast in 1951, and 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.* Several members of genera *Trichosporon* reported to be celulolytic yeast (Stevens and Payne, 1977). Our present work is dedicated on studying the characteristic of celulolytic yeast isolated from soil.

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 (lOg/L), agar (20g/L), 0.2% dichloran solution and streptomycin (100U/L), 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 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) Kurtzman *et al.,* 1998.

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-cc-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 O.I 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 % tanic 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}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 (JEOL, Japan) at 5000 x magnification.

Celulolyitic 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 cellulolyitc activity (Joson and Coronel, 1986). After 5-day incubation celiulolytic ability was determined, the ratio of clearing zone to

colony was calculated. The strain that has highest cellulolytic capacity is then cultivated in 1 % CMC contained media with addition of 0.05 % glucose or CMC alone.

Culture filtrates and enzyme assays.

Yeast culture were centrifuged and successively filtered through Miliopore membrane filter (pore size 0.8 µm and 0.45 (im, type MF, mixed cellulose ester) to remove cell. The filtrate were then used for the source enzymes. The enzymes activity is expressed in unit activity namely µmol glucose equivalent produced ml'" filtrate enzymes minute"[1] (Hatano *et al.*, 1991).

RESULTS

Many celluloytic yeasts belonged to genera of *Debaryomyces, Rhodotorula, Pichia* and *Candida* were isolated from GHNP. Its celulolytic capacity were assayed by growing the culture in the media contained carboxymethyl cellulose as the sole carbon sources (Figure 1).

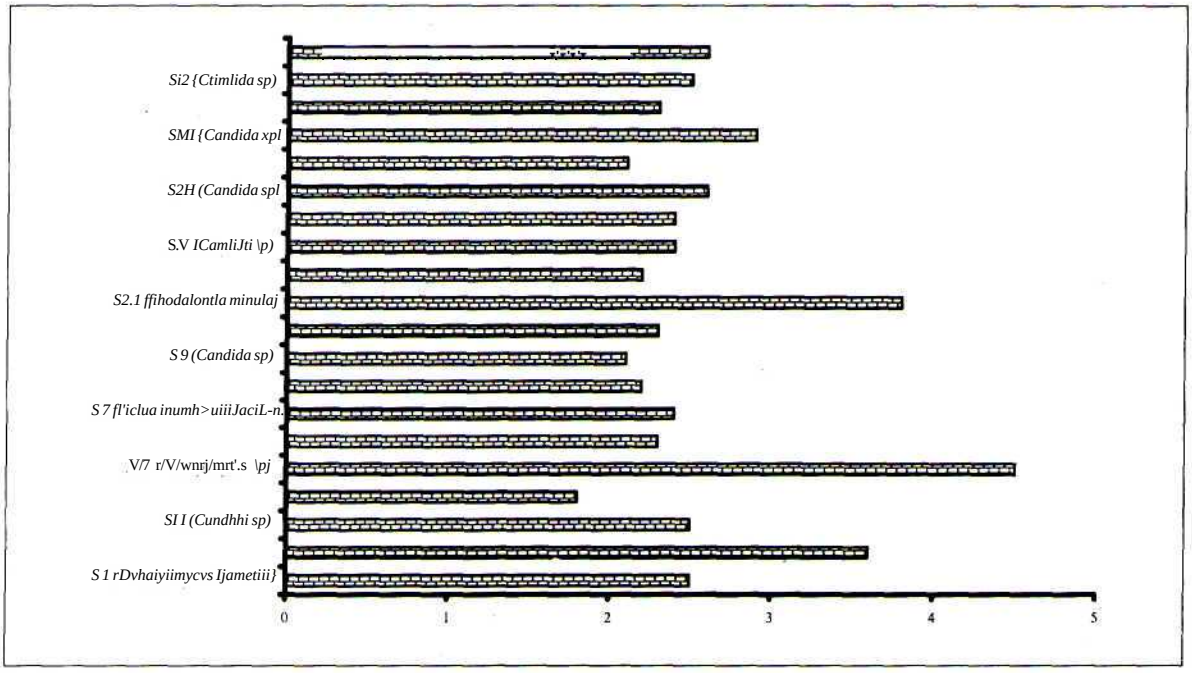
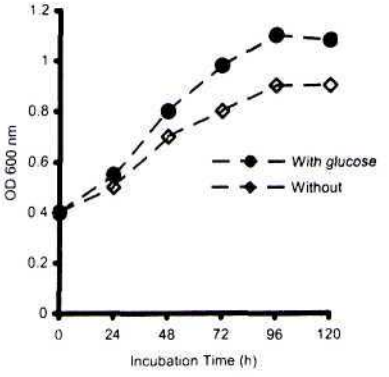


Figure 1. Celulolytic capacity of soil yeast



Carboxymethyl cellulose was rapidly hydrolyzed, the end product was then taken and rapidly converted into cell biomass. Rapid cell growth was encountered after 24 h incubation and static growth achieved after 96 h incubation (Figure 2).

Specific growth rate

Glucose augmentation stimulates rate of cell synthesis as indicated by biomass production achieved when glucose was added (Figure 3).

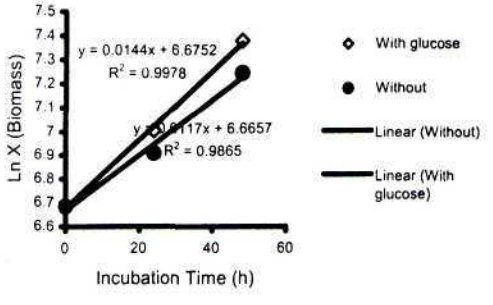


Figure 3. Specific growth rate of *Debaryomyces* sp.Growth of culture

CMCase

CMCase activity was maximum after 3 days incubation. Glucose stimulate cell synthesis and further biomass formed stimulate enzymes synthesis (Figure 4).

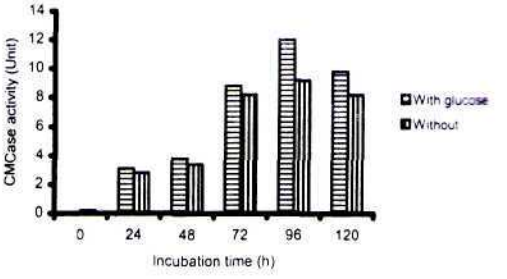


Figure 4. CMCase activity

DISCUSSION

The present work was initiated to study the cellulolytic ability of soil yeast isolated from Gunung Halimun National park. Previously Stevens and Payne (1977) reported *Trichosporor cutaneum* and *T. pulhdans* as cellulolytic yeast. The effort of finding other cellulolytic yeast gained popularity. Our study noted that many soil yeast hydrolyzed CMC implying that they produced extra-cellular enzymes 1,4 P D-endoglucanase. Those soil yeasts belonged to genera of *Candida*, *Rhodothorula, Pichia* and *Debaryomyces.* Its high diversity indicated that they significantly contribute on biodegradation of lignocelluloses material in forest soil. Glucose clearly has significant effect or cell synthesis, however the effect of glucose or, enzymes activity is not consistent.

Steven and Payne (1971) observed that addition of small amount of glucose does not stimulate cellulase and xylanase activity, and they also noted that limitation of glucose by glucose starvation appeared to suppress cell and enzymes synthesis. Cell growth was static after 96 h incubation and enzymes synthesis appeared to be concomitant with cell growth, implying tha: enzymes synthesis and activity is controlled by cell substrate demand.

The mechanism of cellulose hydrolyzes appeared executed by a complex mechanism via multi-enzymes action (Enari, 1983; Gal *et al,*

1997; Soham *et al.*, 1999), and thus the ability of yeast to synthesis complex enzymes of cellulase will determine its survival and adaptability in a complex ecosystem such as forest soil. Yeast could be co-exist with bacteria and fungi in natural environment (Hiroki and Watanabe, 1996; Elberson *et* a/., 2000) and they perform specific metabolism and natural adaptation.

CONCLUSION

Many soil yeast were isolated from soil of Gunung Halimun National park. Some of them were cellulolytic. Common occurrence of soil yeast and their capacity to breakdown polymeric substances implying that they play significant role on biotransformation of organic substances in soil.

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