

Carboxymethyl Cellulose Hydrolyzing Yeast Isolated from South East Sulawesi, Indonesia
Khamir Penghidrolisa Selulosa Karboxymethyl yang Diisolasi dari Sulawesi Tenggara Indonesia

Atit Kanti

Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong Science Centre, Jl.Raya Bogor-Jakarta Km. 46, Cibinong 16911, Indonesia
atityeast@gmail.com

Received: May 2015, **Accepted:** May 2015

ABSTRACT

The objective of study was to isolate, identify and characterize the CMC-ase producing yeast from South East Sulawesi, Indonesia. We isolated 142 strains and obtain 53 strains (37.32%) were CMC-ase producer consist of 26 species residing within 10 genera. *Candida* was the most diverse genus consisting of 15 species. It is important to note that several strains residing within this genus could be candidate for new taxa, among others *Candida* aff. *cylindracea* PL2W1, *Candida* aff. *insectorum* PL3W6, *Candida* aff. *friedrichii* MKL7W3, *Candida* aff. *lessepsii*, *Candida* aff. *tenuis*. Five new candidates for novel species of cellulolytic yeast close to *Yamadazyma mexicana*: were *Yamadazyma* aff. *mexicana* (5 strains). *Pichia*, *Pseudozyma*, *Sporodiobolus*, and *Sporobolomyces* were other cellulolytic yeasts found in South East Sulawesi. It is obvious, that leaf litter was a good source for cellulolytic yeasts. This CMC-ase producing yeasts dominate this biome, and production of extracellular cellulase is critical strategy for such yeast to survive in cellulose rich ecosystem such as leaf-litter. This finding would suggest that yeasts play key role on hydrolyzes of cellulose and important resources for sustainable energy research.

Keywords: cellulolytic yeasts, secondary forest, South East Sulawesi, leaf-litter

ABSTRAK

Penelitian bertujuan untuk melakukan proses isolasi, identifikasi, dan karakterisasi khamir yang mempunyai kemampuan untuk memproduksi CMC-ase dari Sulawesi Tenggara, Indonesia. Sebanyak 142 strain telah diseleksi dan 53 strain merupakan penghasil CMC-ase (37,32%), yang terdiri dari 26 jenis yang tergolong dalam 10 marga. *Candida* merupakan marga yang paling beragam, terdiri dari 15 jenis. Hal penting yang perlu dicatat adalah beberapa strains yang termasuk marga tersebut merupakan kandidat taksa baru, diantaranya *Candida* aff. *cylindracea* PL2W1, *Candida* aff. *insectorum* PL3W6, *Candida* aff. *friedrichii* MKL7W3, *Candida* aff. *lessepsii*, *Candida* aff. *tenuis*. Lima kandidat jenis baru yang merupakan khamir selulolitik yang berkerabat dekat dengan *Yamadazyma mexicana*: adalah *Yamadazyma* aff. *mexicana* (5 strains). *Pichia*, *Pseudozyma*, *Sporodiobolus*, dan *Sporobolomyces* adalah khamir selulolitik lain yang ditemukan di Sulawesi Tenggara. Berdasarkan dari hasil yang didapat, sangat jelas bahwa serasah merupakan sumber yang potensial untuk mendapatkan khamir selulolitik. Khamir penghasil CMC-ase ini mendominasi bioma tersebut, dan produksi dari selulase ekstraseluler merupakan strategi kunci agar dapat bertahan hidup di ekosistem yang kaya selulosa seperti serasah. Dari penemuan ini maka dapat diambil kesimpulan bahwa khamir memegang peranan penting pada proses hidrolisis selulosa dan merupakan sumber penting bagi penelitian energi terbarukan.

Kata Kunci: khamir selulolitik, hutan sekunder, Sulawesi Tenggara, serasah

INTRODUCTION

Up to date ecological studies of yeast from natural habitats have been conducted extensively mostly in temperate regions (Wang & Bai 2004; Butinar *et al.* 2005). Taxonomy and ecology data indicate a need for additional studies in tropical ecosystems, particularly in Asia (Nakase *et al.* 2006; Takashima *et al.*

2012). Indonesia is a tropical nation comprised of over 17,000 islands is rich in biodiversity, having unique flora and fauna (Allend 2009), and presumably microbes as well. Rifai 1995 estimated Indonesia has more than 200,000 species of fungi. However, little information on species diversity of Indonesia indigenous yeast and yeast-like fungi has been published. Studies of Indonesian yeasts primarily related

to their role in fermented foods (Abe *et al.* 2004; Kuriyama *et al.* 1997). Early studies of yeast from natural environment in Indonesia include Deinema in 1961, who found *Candida bogoriensis* from the surface of leaves of the flowering shrubs *Randia melleifera* (Rubiaceae) in Bogor. In recent years, more studies have been performed to explore yeast diversity in Indonesia (Sjamsuridzal *et al.* 2010; Sudiana & Rahmansyah 2002).

While microorganisms are very important sources for bioindustry however few study conducted to assess the important of forest as genetic resources for many interest. Cellulase are primarily obtained from cellulolytic fungi: *Trichoderma reseei*, *T. viride*, *T. lignorum* (Fahrurrozi *et al.* 2010), *T. koningi*, *Chrysosporium lignorum*, *C. prunosum*, *Fusarium solani*, *Neurospora crassa*, *Aspergillus* (Golddbeck *et al.* 2013a), *Penicillium*, *Acremonium strictum* (Golddbeck *et al.* 2013b), including that from several group of cellulolytic bacteria: *Cellulomonas*, *Cellulosimicrobium cellulans* (Lo *et al.* 2009) *Clostridium clariflavum*, *Bacillus* group, *Flavobacterium*, and *Paenibacillus* (Zhanga *et al.* 2013), and *Fermicutes* and *Actinomycetes* group (Zainudin *et al.* 2013). On the other hand the role of yeast on biodegradation of cellulose is few explored, particularly using yeast from tropical forest (Jimenez *et al.* 2007; Kanti *et al.* 2014).

Sulawesi has high biodiversity, and reported to have high biodiversity of flora (Cannon 2007) and fauna (Koch 2011). At the microbial diversity level, further study is needed to verify the species richness of this area, particularly cellulolytic yeast. We evaluated two areas in South East Sulawesi that have different covering vegetation. Leaf litter, soil and leaf surfaces are common habitats for yeast (Takashima *et al.* 2002; Chang *et al.* 2012). This paper is concerned with the isolation of cellulolytic yeast from natural habitats in Southeast Sulawesi, Indonesia and their phylogenetic affiliation based on partial 26S rDNA and ITS1/5.8S/ITS2 region.

MATERIALS AND METHODS

Leaf and leaf litter samples were aseptically collected from two sites that differ in elevation, forest type, and land use type.

Papalia Protected Forest is a tropical lowland monsoon wet forest dominated by evergreen tree, with a high humidity and high rain fall. Papalia, located in South Konawe, GPS S 04° 13' 526", E 122 44 301", having altitude <1000 m asl. Whereas Mekongga Protected Forest covers lowland and upland rain forest sites included hill forest, montane forest with elevation gradient ranges from 100 m to 2500 m asl. Land use type is dominated by cocoa plantations. Mekongga rain forest is located near Tinukari, North Kolaka, GPS S 03° 38' 085", E 121 04 311". Leaf and leaf litter sampling were conducted in 2009 and 2010. Six leaves and 6 leaf litter samples were collected in Papalia, and six leaves and 10 leaf litter were collected from Mekongga Protected Forest.

Yeasts were isolated from leaf, leaf litter, and soil. One g of soil or leaf litter was added to 25 mL of saline/Tween (0.85% NaCl, 0.01% Tween 80, v/v) buffer in a 7 oz Whirl-Pak filter bag (Cat. B01385WA, Nasco, Salida, CA, USA) and shaken to suspend the microbes. The bag had two separated compartments which allowed separation of suspension from debris. Two hundreds μ L of suspension were plated on Rose Bengal Chloramphenicol Agar (RBCA) plates supplemented with chloramphenicol. Leaves were plated using two methods, washing and direct plating. For washing, leaves were added to 10 mL of saline/Tween buffer in a 7 oz. Whirl-Pak filter bag and processed as detailed previously. Aliquots of 200 μ L and 50 μ L of these samples were plated on RBCA, which prevents growth of bacteria and slows down the spread of molds. For direct plating, the leaf and leaf litter were weighed and cut into small pieces of about 2cm². The leaf and leaf litter were washed with 30 ml of sterile distilled water and vortexed for 5 min. Washed materials were placed directly onto RBCA plates.

Ballistospore-producing yeasts were isolated from leaves using the ballistospore-fall method. Briefly, aseptically collected segments of leaves were attached to the underside of a Petri dish lid using Vaseline, and the plate was incubated lid-side up. Ballistospores ejected onto the surface of the agar germinated, and yeasts were cultivated. Incubation of the plates

was done for 5 days at room temperature. Strain purification was done at least twice by selecting the different type of yeast colony and plated on potato dextrose agar (PDA, CM0139, OXOID) at room temperature.

Yeast DNA template was prepared from freshly-grown cells on the PDA plate and used for colony PCR. Five μ L of lysed yeast cell suspension was used for PCR amplification of the partial 26S rDNA sub unit with primers NL1 and NL4 (O'Donnel, 1992), using GoTaq master mix (Promega, M7122). PCR products were visualized on 2% agarose and sequenced with both primers using Big Dye terminator v3.1. Cycle Sequencing Ready Reaction Kit (Applied Biosystems) following the manufacturer's instructions. The partial 26S sequences determined in this study were compared to those in the EMBL/GenBank/DDBJ databases using the nucleotide Basic Local Alignment Search Tool (BLASTn). The ITS1/5.8S/ITS2 region of selected strains was also amplified with primers ITS1 and ITS4 Kurtzman and Robnett, 1998 when species identifications were ambiguous.

Sequences were aligned using CLUSTALX (Altschul *et al.* 1997). The distance matrix for the aligned sequence was calculated using the two-parameter methods of Kimura, 1980. The neighbor-joining (NJ) method & Nei (1987) was used to construct all phylogenetic trees.

Cellulolytic yeast is defined as yeasts grow on CMC as the sole carbon sources, and produce CMC-ase. To obtain the cellulolytic each yeast was grown on CMC agar containing 3 g L⁻¹ yeast extract, 5 g L⁻¹ peptone, 10 g L⁻¹ CMC, K₂HPO₄ 5 g L⁻¹, (NH₄)₂SO₄ 0,5 g L⁻¹, MgSO₄.H₂O 0,2 L⁻¹, FeCl₃.6H₂O 0,01 g L⁻¹, MnSO₄.H₂O 0.001 g L⁻¹, 20 g L⁻¹ agar (pH 6.2 \pm 0.2) and incubated for 5 days at room temperature. Cellulolytic yeast was determined by pouring 2 mL congo red 1 M solution into grown colonies, and kept for 10 minutes. Observing clear zone was done by pouring the congo red solution and replaced with NaCl 0.1N. Cellulolytic yeast was indicated by formation of clear zone surrounding colonies and the ratio between clear zone divided by colony's size indicating cellulolytic capacity

Yeast isolates were preserved by two methods, in 20 % glycerol solution at -80°C,

and by lyophilization. Yeasts were deposited in the Indonesian Culture Collection (InaCC, www.biologi.lipi.go.id) at the Indonesian Institute of Sciences, Research Center for Biology; the Forest Microbes Collection (FORDACC, http://fordamof.org/) at the Forestry Research and Development Agency, Indonesian Ministry of Forestry; and the Phaff Yeast Culture Collection, Department of Food Science and Technology, University of California Davis (UCDFST, phaffcollection.ucdavis.edu).

RESULTS

Diversity of CMC-ase producing yeast

Numerous of yeasts were isolated from two sites (Mekongga and Papalia) in South East Sulawesi. The cellulolytic yeast was defined as yeast grow on CMC used as the sole carbon sources and produce CMC-ase (Endoglucanases EG1, EC. 3.2.1.4) hydrolyze soluble, substituted celluloses such as Carboxymethyl Cellulose (CMC) by attacking the carbohydrate chain (1-4, β glucosidic bond) internally and randomly. This can be visualized by culturing yeast both on liquid and solid media contain CMC. Formation of clearing zone on CMC-media poured with 1 % congo red and production of CMC-ase were used to screen cellulolytic yeast. We obtained numerous species of yeasts (Figure 1). Of 142 strains tested 43 strains were cellulolytic yeasts consist of 10 genera and 26 species, of whose *Candida* was the most diverse genus consisting of 15 species. *Sporidiobolus rumiaes* were most prevalent CMC-ase producing yeast from South East Sulawesi represented by 10 isolates (Figure.1). *Candida intermedia*, *Pseudozyma aphidis* and *Asterotremella humicola* were second predominating CMC-ase producing yeast.

The most frequently isolated yeast genus from Mekongga and Papalia was *Candida*. *Candida* was isolated from both locations and sample types. *Candida* is a polyphyletic genus, with species placed in 14 families within the class *Saccharomycotina*. In fact, over 400 of the 1600 known species of yeasts have been placed in the genus *Candida* (Fell & Boekhout 2011). Due to its taxonomic diversity, it is not surprising that *Candida* is ecologically diverse also, occupying niches including human infections,

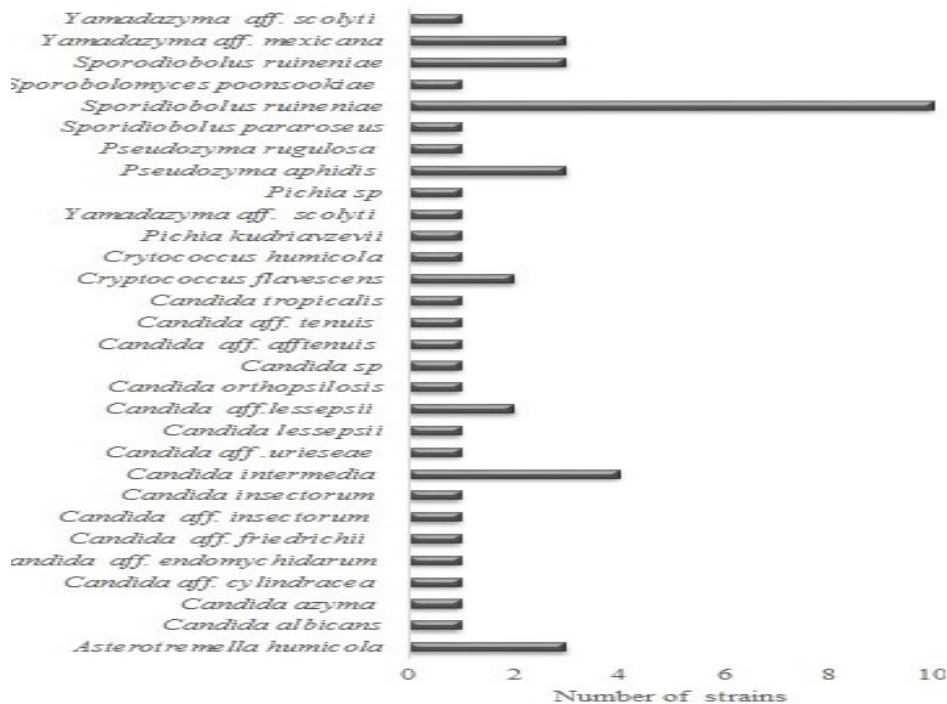


Figure 1. Diversity of CMC-ase producing yeast from South East Sulawesi

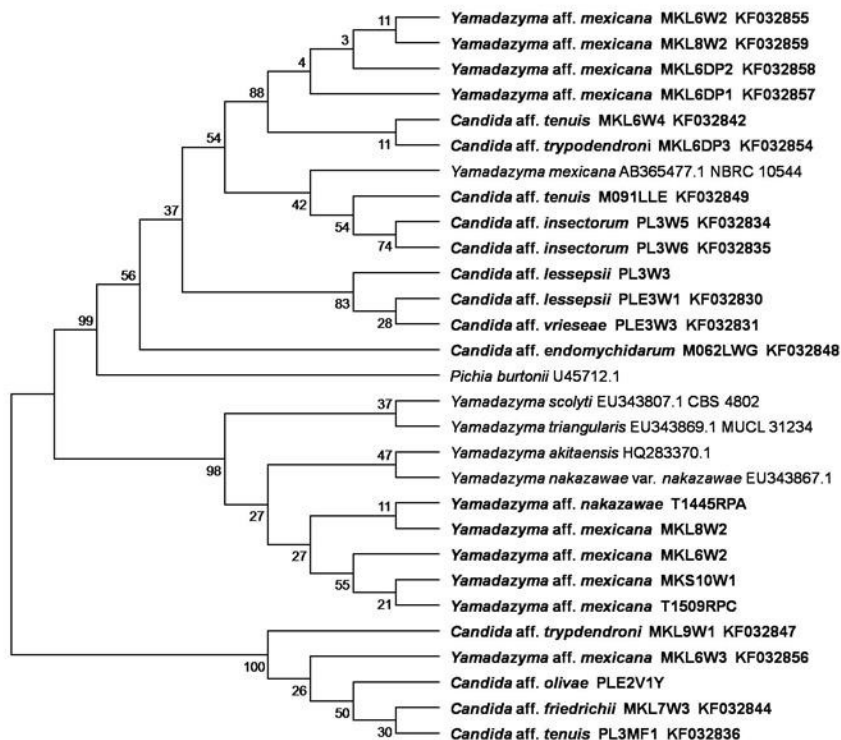


Figure 2. Phylogenetic placement of yeast from South East Sulawesi within *Yamadazyma* clade based on D1/D2 region of the nLSU rDNA sequences

soil (Amprayna *et al.* 2012), insect frass, fruit (Wilson & Chalutz 1989), and many other habitats.

Some yeasts isolated in this study are ecologically important for sustainable agriculture (Fracchia *et al.* 2003). *Sporiodiobolus pararoseus* shows promise for biocontrol of the fungal plant pathogen *Botrytis cinerea* (Huang *et al.* 2012).

Novel taxa of CMC-ase producing yeast

Comparison of the D1/D2 region of LSU rDNA data showed 14 strains belonging to 11 different species had homology values less than 99%, indicating that they may be novel species. A number of potential novel species were collected in this study, as indicated by 1% or higher sequence divergence from previously known species in the D1D2 domain. The results of ITS sequence analysis of these isolates confirmed that they are likely novel species. Our molecular analysis revealed that these yeast isolates are phylogenetically diverse and distributed

within the phyla *Ascomycota* (*Yamadazyma* clade and *Metschnikowia* clade) (Figure 2 and Figure 3).

The novel species candidates were mostly residing within genus *Candida* (13 species: *Candida* aff. *cylindracea* PL2W1, *Candida* aff. *insectorum* PL3W6, *Candida* aff. *friedrichii* MKL7W3, *Candida* aff. *lessepsii* PLE3W1, MKL7W4, *Candida* aff. *tenuis* PL3DP3, MKL6W4. We isolated 5 strains of cellulolytic yeast close to *Yamadazyma mexicana*: *Yamadazyma* aff. *mexicana* MKL6DP1, MKL6DP2, MKL8W2, MKL6W2, MKL6W1), and our study reveal detection of many undescribed yeast from Indonesia (Figure.4). Further study is needed to describe the novel taxa found in this study.

Phylogeography of CMC-ase producing yeast

Sample sources were collected from secondary forest in Mekongga and Papalia, South East Sulawesi. Both places harbor numerous species of Cellulolytic yeast (Figure. 4 and 5). There were 15 species including 6 candidates for novel species (3

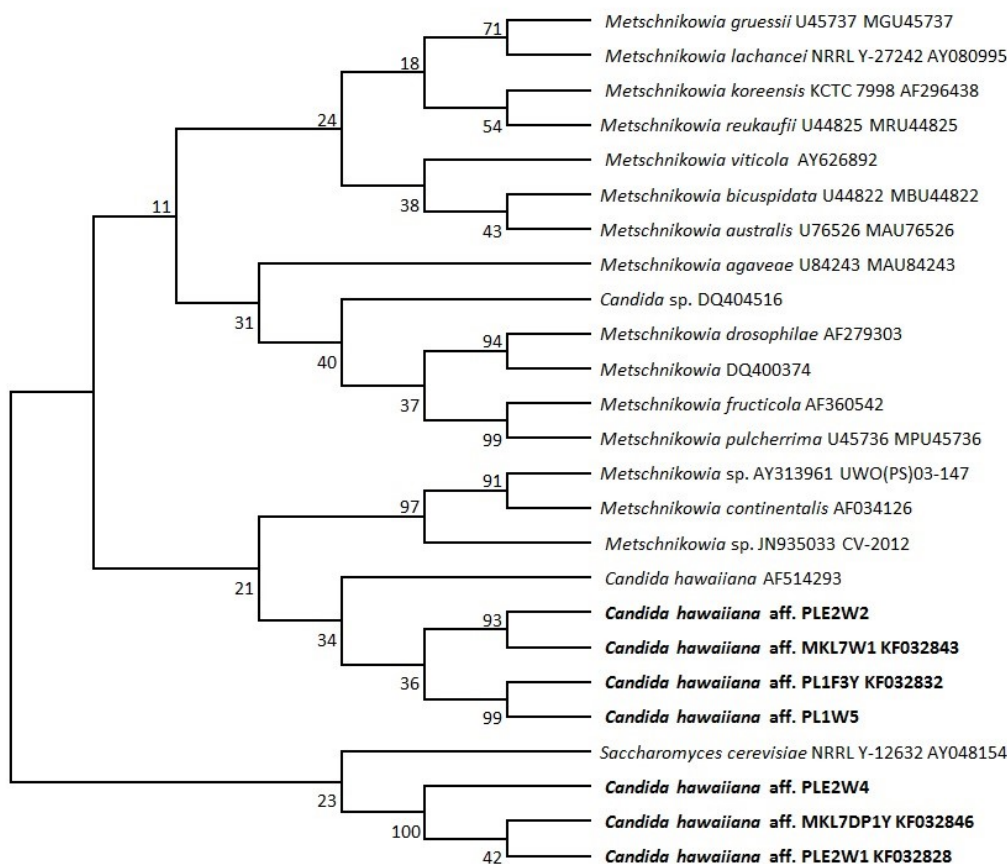


Figure 3. Phylogenetic placement of yeast from South East Sulawesi within *Metschnikowia* clade based on D1/D2 region of the nLSU rDNA sequences

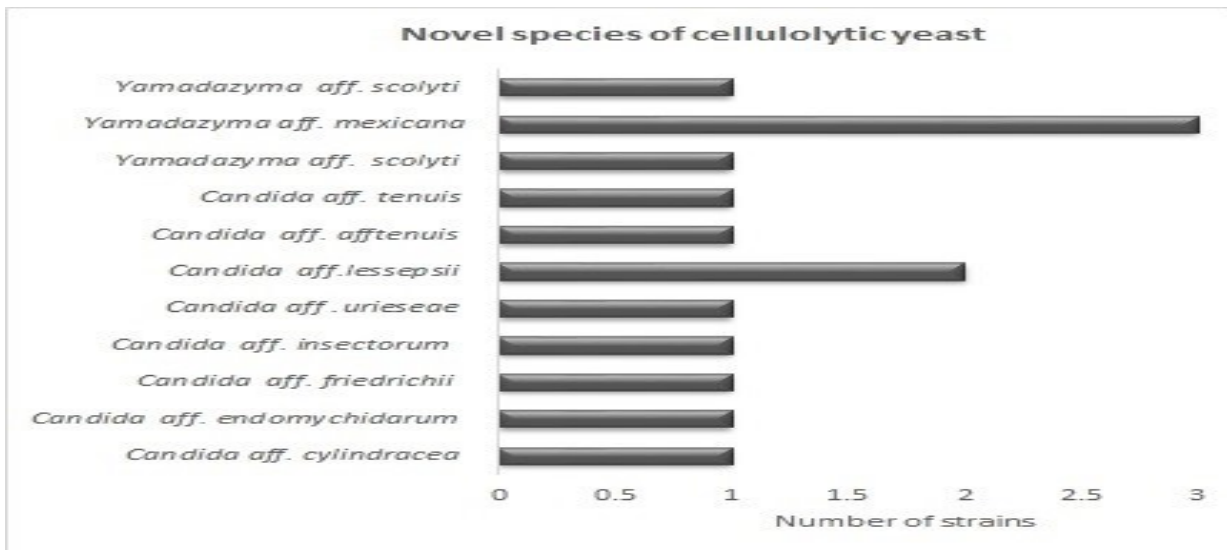


Figure 4. Candidate for novel species of CMC-ase producing yeast

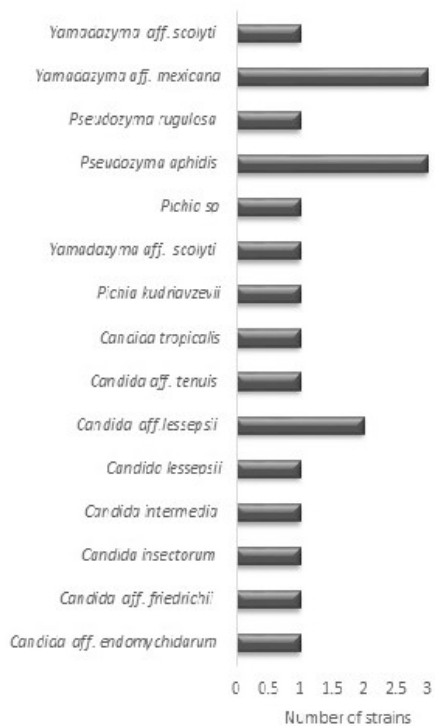


Figure 5. CMC-ase producing yeast isolated from Mekongga

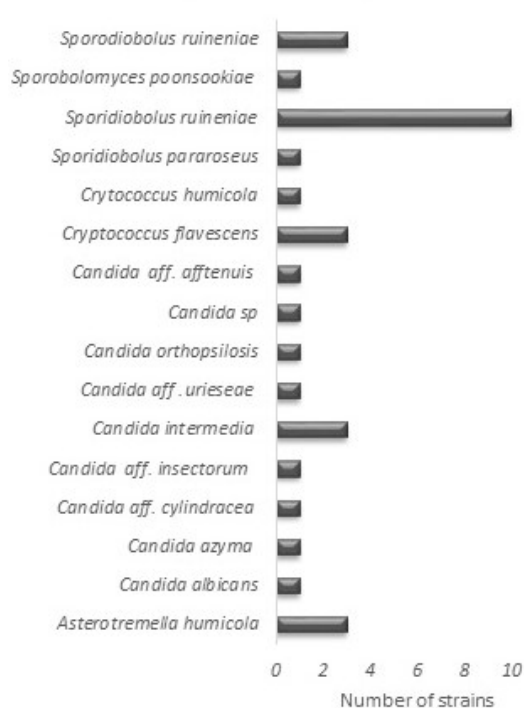


Figure 6. Cellulolytic yeast isolated from Papalia

strains of *Yamadazyma aff. scolyti*, *Y. aff. mexicana*, *Candida aff. tenuis*, *C. aff. lessepsii*, *C. aff. friedrichii*, and *C. aff. endomychidarum*) Figure. 4. Eleven species including 3 candidates for novel species of cellulolytic yeast (*Candida aff. tenuis*, *C. aff. insectorium*, *C. aff. cylindracea*) were found in Papalia (Figure. 6). *Yamadazyma* were only isolated

from Mekongga, and *Asterotremella* and *Sporidiobolus* were only cultivated from Papalia.

CMC-ase yeast producer on Litter

Litter are good sources for cellulolytic yeast, as indicated by numerous cellulolytic yeasts obtained from litter collected both from

Mekongga (Figure. 5) and Papalia (Figure. 6). This study supports other studies that concluded that leaf litter and plant leaf surfaces are good sources of a diversity of yeast cultures for research (Wang *et al.* 2004, Nasase *et al.* 2002, Fungsin *et al.* 2002).

CMC-ase yeast producer on leaf

Seven species of cellulolytic yeasts were isolated from Papalia leaf, and none was from Mekongga. *Sporodiobolus* were common genera, and *Sporodiobolus ruineneniae* and *Candida intermedia* were dominant in leaf (Figure. 7 ,8 and 9).

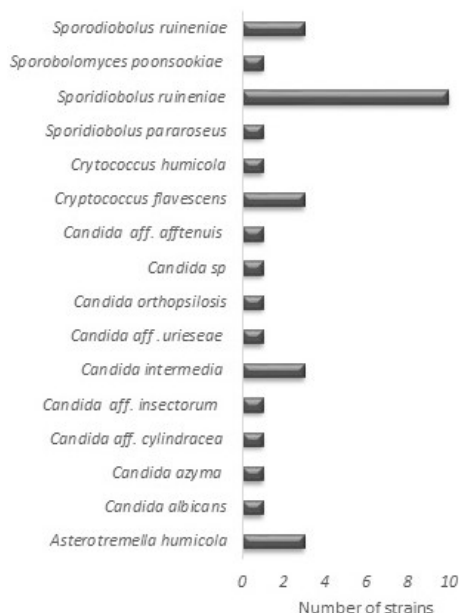


Figure 7. Mekongga CMC-ase producing yeast from litter

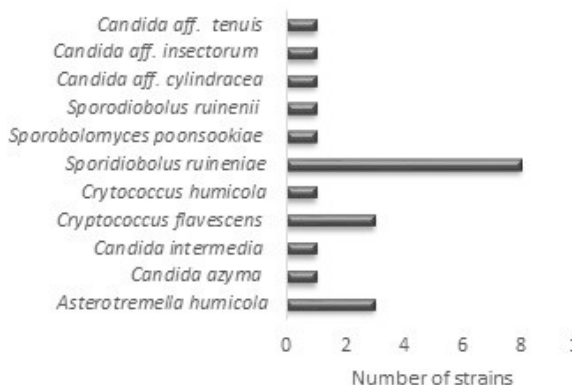


Figure 8. Papalia CMC-ase producing yeast from litter

Sporodiobolus ruineneniae seem to be a tropical yeast because only found in tropical region. It was first found in Indonesia by Ruinen in (1963) then it was also isolated in Thailand but not in the temperate zone (Nakase *et al.* 2006).

DISCUSSION

Little information was previously available about yeasts on the island of Sulawesi, Indonesia, one of the five largest islands that makes up this richly biodiversity and biogeographically significant region (Sihotang *et al.* 2012). We found a broad taxonomic diversity of cellulolytic yeast species from this exploratory survey. Because plant surfaces and leaf litter have been sampled extensively (Amprayna *et al.* 2012; Wilson & Chalutz, 1989), novel taxa were not expected. However, numerous potentially novel species of cellulolytic yeast were obtained. Novel strains of known species were obtained, expanding the known geographic and habitat range of these known species.

A variety of ascomycetous and basidiomycetous yeasts were cultivated. Basidiomycetous yeasts are more likely to utilize a broader range of carbon sources than ascomycetes, and have been cultivated more frequently from low-nutrient habitats such as leaf surfaces (Joo *et al.* 2008). The most frequently cellulolytic yeast isolated from South East Sulawesi were *Yamadazyma*, *Pseudozyma* and *Candida*. *Candida* was isolated from both locations and

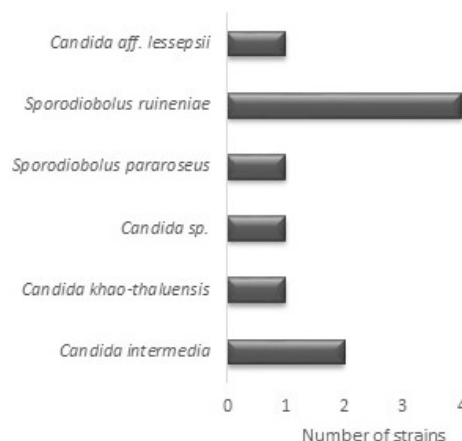


Figure 9. CMC-ase producing yeasts obtained from Papalia leaf

ubiquitous on litter. *Candida* is a polyphyletic genus, with species placed in 14 families within the class Saccharomycotina. In fact, over 400 of the 1600 known species of yeasts have been placed in the genus *Candida* (Junyapate *et al.* 2013). Due to its taxonomic diversity, it is not surprising that *Candida* is ecologically diverse also, occupying niches including human infections, soil, insect frass, fruit. Important applications using *Candida* species include agent for bioremediation, *Candida catenulata*, and biofertilizer, *Candida tropicalis* HY. *Yamadazyma*, *Pseudozyma* and *Candida*, isolated repeatedly in this study, is a well known species having wide distribution and having high xylose transport capacity.

This study supports other studies that concluded leaf litter and plant leaf surfaces are good sources of a diversity of yeast cultures for research (Wang & Bai. 2004; Altschul *et al.* 1997). We found leaf litters are good sources for cellulolytic yeast. The strains isolated in this study were deposited in Indonesian Culture Collection (InaCC). Isolation of numerous species of yeasts include novel taxa reaffirm that South East Sulawesi are rich in biodiversity of flora, fauna and microorganism, and potential genetic resources for sustainable development.

ACKNOWLEDGMENTS

Yeasts used in this study were isolated and identified as part of a collaborative project between the University of California Davis and the Government of the Republic of Indonesia, funded by Grant Number U01TW008160 from the US National Institutes of Health Fogarty International Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Fogarty International Center or the National Institutes of Health, the Office of Dietary Supplements, the National Science Foundation, the Department of Energy, or the Department of Agriculture. The author acknowledge Kyria Boundy-Mills, Ph.D and Dr. Irnayuli Sitepu for manuscripts editing. Yeni Yuliani for laboratory work.

REFERENCES

- Abe, A., N. Sujaya, T. Sano & K. Asano. 2004. Microflora and Selected Metabolites of Potato Pulp Fermented with an Indonesian Starter Ragi Tapé, *Food Technol. Biotechnol.* 42 (3): 169-173.
- Allend, GR. & MV. Erdmann. 2009. Reef fishes of the Bird's Head Peninsula, West Papua, Indonesia," *Check List.* 5: 587-628.
- Altschul, SF., TL. Madden, AA. Schäffer, J. Zhang, Z. Zhang, W. Miller & DJ. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research.* 25: 3389-3402.
- Amprayna, K., MT. Rosea, M. Kecskés, L. Pereg, HT. Nguyend, & IR. Kennedy. 2012. Plant growth promoting characteristics of soil yeast *Candida tropicalis* HY and its effectiveness for promoting rice growth. *Applied Soil Ecology.* 6: 295-299.
- Butinar, L., S. Santos, I. Spencer-Martin, A. Oren, & N. Gunde-Cimerman. 2005. Yeast diversity in hypersaline habitats. *FEMS Microbiology Letter*, vol. 244: 229-234.
- Cannon, C. 2007. Interim Report. The Study on Arterial Road Network Development Plan for Sulawesi Island and Feasibility Study on Priority Arterial Development for South Sulawesi Province.
- Chang, F.C., Y.R. Liu, S.F. Chen, G.I. Naumov, E.S. Naumova & C.F. Lee. 2012. Five novel species of the anamorphic genus *Candida* in the *Cyberlindnera* clade isolated from natural substrates in Taiwan. *Antonie van Leeuwenhoek.* 102: 9-21.
- Dewi K. & Hasegawa. H. 2012. Two new species of *Syphacia* (Nematoda: Oxyuridae) in endemic murid rodents from Sulawesi, Indonesia. *J. Helminthology.* 2 (12): 1-9.
- Fahrurrozi, S. Ratnakomala, T. Anindyawati, P. Lisdiyanti, & E. Sukara. 2010. Rapid Assessment of Diverse Trichoderma Isolates of Indonesian Origin for Cellulase Production, *Annales Bogorienses.* 14 (1): 39-43.
- Fungsin, B., M. Hamamoto, V. Arunpairojana, V., Sukhumavasi, J., Atthasampunna, P., &

- Nakase, T. 2002. *Kockovaella barringtoniae* sp. nov., a new basidiomycetous yeast species isolated from a plant leaf collected in a tropical rain forest in Thailand. *International Journal Systematic Evolutionary Microbiology*. 52 : 281-4.
- Goldbeck, R., M.M. Ramos, G.A.G Pereira & F. Maugeri-Filho. 2013. Production of cellulase by a newly isolated strain of *Aspergillus sydowii* and its optimization under submerged fermentation. *International Biodeterioration & Biodegradation*. 78: 24-33.
- Goldbeck, R., M.M. Ramos, G.A.G. Pereira, & F. Maugeri-Filho. 2013. Cellulase production from a new strain *Acremonium strictum* isolated from the Brazilian Biome using different substrates, *Bioresource Technology*. 128: 797-803.
- Jiménez, M., A.E Gonzáles, M.J. Martínez, A.T. Martínez & B.E. Dale. 2002. Screening of yeasts isolated from decayed wood for lignocellulose-degrading enzyme activities, *Mycol. Res*. 9 (11): 1299-1302.
- Joo, H.S., P.M. Ndegwa, M. Shoda, & C.G. Phae. 2008. Bioremediation of oil-contaminated soil using *Candida catenulate* and food waste. *Environmental Pollution*. 156: 891- 896.
- Junyapate, K., S. Jindamorakot, & S Limtong S. 2013. *Yamadazyma ubonensis* f.a., sp. nov., a novel xylitol-producing yeast species isolated in Thailand. *Antonie Van Leeuwenhoek*. DOI 10.1007/s10482-013-0098-8.
- Kang, S. W., Y. S. Park, J.S. Lee, S.I. Hong and S.W. Kim. 2004. Production of Cellulases and Hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. *Bioresource Technology*. 91: 153-156.
- Kanti, A. , E. Sukara, K. Latifah, N. Sukarno, & K. Boundy-Mills. 2013. Indonesian oleaginous yeasts isolated from *Piper betle* and *P. nigrum*. *Mycosphere*. 4 (5): 1015-1026.
- Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal Molecular and Evolution*. 16: 111-120.
- Koch, A. 2011. The Amphibians and Reptiles of Sulawesi : Underestimated Diversity in a Dynamic Environment. *Biodiversity Hotspots* 5: 383-404.
- Kuriyama, H., D. Sastraatmadja, Y. Igosaki, K. Watabane, A. Kanti, and T. Fukatsu. 1997. Identification and Characterization of Yeast isolated from Indonesian fermented food. *Mycoscience* 38: 441-445.
- Kurtzman, C.P. & C.J. Robnett. 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Leeuwenhoek*. 73: 331-71.
- Lo, Y-C., G.D. Saratale, W-M. Chen, M-D. Bai & J -S Changa. 2009. Isolation of cellulose-hydrolytic bacteria and applications of the cellulolytic enzymes for cellulosic biohydrogen production, *Enzyme and Microbial Technology*. 44: 417-419.
- Nakase, T., S. Jindamorakot, S. Am-In, S., W. Potacharoen, & M. Tanticharoen. 2006. Yeast biodiversity in tropical forest of Asia, In *Biodiversity and ecophysiology of Yeast*, Rosa, C., Peter., G., Berlin Heidelberg, Ed. Springer-Verlag: 441-60.
- Nakase, T., S. Suzuki & M. Takashima. 2002. *Bullera taiwanensis* sp nov and *Bullera formosensis* sp nov, two new ballistoconidium-forming yeast species isolated from plant leaves in Taiwan. *Journal Genetic Applied. Microbiology*. 48: 345-355.
- Nakase, T., S. Suzuki, M. Takashima, M. 2002. *Bullera taiwanensis* sp nov and *Bullera formosensis* sp nov, two new ballistoconidium-forming yeast species isolated from plant leaves in Taiwan. *J. Gen. Appl. Microbiol.*, 48: 345-355.
- O'Donnel, K. 1992. Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Current Genetic*. 22: 213-220.
- Rifai, MA. 1995. The biodiversity of Indonesian microorganisms. in *Proc. of UNESCO Regional Workshop on Culture Collection of Microorganisms in Southeast Asia*, Yogyakarta.
- Sihotang, VBL., EA. Widjaja & D. Potter. 2012. Medicinal plant knowledge of Tolaki and Toraja in Tinukari Village and its surrounding in : *Proc. Strategies and*

- Challenges on Bamboo and Potential Non Timber Forest Products (NTFPs) Management and Utilization*, Bogor.
- Sjamsuridzal, W., A. Oetari, A. Kanti, R. Saraswati, C. Nakashima, Y. Widyastuti & A. Katsuhiko. 2010. Ecological and Taxonomical Perspective of Yeast in Indonesia. *Microbiology Indonesia*. 4: 49-57.
- Sudiana, IM. & M. Rahmansyah. 2002. *Species and functional diversity of soil microflora at Gunung Halimun National Park*. Jakarta : BCP-JICA. LIPI Press.
- Takashima, M., T. Sugita, BH. Van, M. Nakamura, M. Endoh & M. Ohkuma. 2012. Taxonomic richness of yeast in Japan within subtropical and cool temperate areas. *Plos ONE* [Online]. 7(11). Available: e50784, 2012. doi: 10.1371/journal.pone.0050784.
- Wang, QM. & FY. Bai. 2004. Four new species of the genus *Sporobolomyces* from plant leaves. *FEMS Yeast Research*. 4: 579-586.
- Wilson, CL., & E. Chalutz. 1989. Postharvest biological control of *Penicillium* rots of citrus with antagonistic yeasts and bacteria. *Sc. Hortic*. 40: 105-112.
- Zainudin, MHM., MA. Hassan, M. Tokura & Y. Shirai. 2013. Indigenous cellulolytic and hemicellulolytic bacteria enhanced rapid co-composting of lignocellulose oil palm empty fruit bunch with palm oil mill effluent anaerobic sludge. *Bioresource Technology*. 147: 632-635.
- Zhanga, Q., M. Tian, L. Tang, H. Li, W. Li , J. Zhang, H. Zhang & Z. Mao. 2013. Exploration of the key microbes involved in the cellulolytic activity of a microbial consortium by serial dilution. *Bioresour- ce Technology*. 132: 395-400.