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DIVERSITY OF XYLOSE ASSIMILATING YEAST FROM THE ISLAND OF ENGGANO, SUMATERA, INDONESIA [Keragaman Khamir Pengguna Xilose yang Diisolasi dari Pulau Enggano, Sumatera, Indonesia]

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ABSTRAK

Khamir yang secara alami terdapat di alam merupakan salah satu sumber daya genetik penting yang banyak digunakan untuk kepentingan industri. Meskipun eksplorasi keanekaragaman hayati Enggano telah dilakukan sejak tahun 1944, akan tetapi tidak ada laporan tentang keragaman khamir dari pulau ini. Karena banyak digunakan untuk perkembangan industri, khamir yang dapat menggunakan xilose merupakan target penelitian bagi banyak ilmuwan. Penelitian ini bertujuan untuk mengisolasi khamir pengguna xilose dari Pulau Enggano. Sampel dikumpulkan dari berbagai sumber yang meliputi tanah, sampah daun, kayu busuk, buah dan sedimen. Teknik kultur pengayaan xilosa dilakukan untuk menumbuhkan khamir pengguna xilosa. Aplikasi metoda yang bervariasi digunakan untuk mengisolasi khamir yang meliputi pengenceran, *ballistopore falling*, isolasi langsung, dan filtrasi dengan menggunakan membran. Dua ratus isolat, dimana 76 strain (38%) merupakan khamir pengguna xilosa yang sebagian besar termasuk ke dalam marga *Candida* yang terdiri dari *C. insectorum*, *C. tropicalis*, *C. boidinii*, *C. pseudolambic*a, *C. yuanshanica*, *C. silvae*, *Cyberlindnera saturnus*, *Williopsis saturnus* dan *Sporobolomyces poonsookiae*. Satu strain *Candida* sp. yang diisolasi dari tanah, dapat dikelompokkan sebagai calon jenis baru berdasarkan analisis urutan gen dari domain D1 / D2. Studi ini mengkonfirmasi bahwa survei keragaman khamir sangat penting untuk mendapatkan sumber daya baru genetik untuk kepentingan industri serta untuk studi taksonomi.

Kata kunci: Enggano, khamir pengguna xilosa, *Candida.*

ABSTRACT

Naturally occurring yeasts (wild type) are important genetic resources for many industrial interests. Though biodiversity exploration of Enggano has been done since 1944, but there is no report for yeast diversity from this island. Due to its industrial interest, xylose fermenting yeast is target for many scientists. This present study aims to isolate xylose assimilating yeast from island of Enggano. The samples were collected from varying sources included soil, leaf litter, decay wood, fruit and sediment. Xylose enrichment culture technique was performed to enrich xylose fermenting yeast. While for the isolation of yeast several methods which include dilution, ballistopore falling, direct isolation, membrane filtration were done. Of 200 isolates, 76 strains (38 %) were xylose assimilating yeast which mostly belong to *Candida* which include *C. insectorum*, *C. tropicalis*, *C. boidinii*, *C. pseudolambica, C. yuanshanica*, *C. silvae*, *Cyberlindnera saturnus*, *Williopsis saturnus* and *Sporobolomyces poonsookiae*. One strain *Candida* sp. that was isolated from soil could be assigned as a candidate of novel species on base on its morphological and biochemical characteristics along with analyses of gene sequence from D1/D2 domain. This study confirms that yeast survey is very important to obtain new genetic resources for industrial interest as well as for taxonomic study.

Key words: Enggano, xylose assimilating yeast, *Candida.*

INTRODUCTION

When Dr. W. J. Lütjeharms explored Enggano in 1936, he collected some plants and deposited as herbaria at Buitenzorg (now Herbarium Bogoriense) and Leiden (Docters van Leeuwen, 1940). Since then biodiversity exploration was started which contribute to the list of flora and fauna of Enggano (Jakl, 2008; Grismer *et al*., 2014). However, no report was for microorganisms. Yeast, together with animal and plant play important roles on biogeochemical cycle which sustain live on Earth (Rastetter, 2011). Though yeasts commonly occupy environment with excessive organic substances, but yeast uses a variety of carbon sources from complex to simple sugar (Ejiofor *et al*., 1996). The contribution of yeast is also significant in live cycle of insect pollinator (Siepel *et al*., 2005).

Recently, yeasts have been exploited for many industrial interests which include biofuel production, pharmaceutical, agrochemicals and others. Soil is important microbial resources (Sláviková *et al*., 2002). To exploit the potential of yeast, the most important step is to isolate, identify and study their physiology (de Souza *et al*., 2012). To obtain specific yeast that has unique metabolism is through cultures enrichment with target carbon sources (Pan *et al*., 2009). Few yeast are able to ferment xylose into ethanol (Zhang and Geng, 2012). Xylose is produced from hydrolyses of hemicellulose that is abundant in nature (Rodrussamee *et al*., 2011). Isolation of xylose fermenting yeast is of interest to many scientists (Ryabova *et al*., 2003). Xylose augmentation succeeds to enrich xylose fermenting yeast (Ryabova *et al*., 2003) (Ishchuk *et al*., 2008).

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The ability of wild-type of yeast *Hansenula polymorpha* to produce ethanol from xylose reaffirm the important of yeast exploration from natural resources, as shown by Ryabova *et al*. (2003). They showed that the thermotolerant methylotrophic yeast *Hansenula polymorpha* ferment xylose, glucose, and cellobiose to ethanol higher than well-known xylose fermenting yeast *Pichia stipitis* at 37°C, whereas the well-known xylose-fermenting yeast *Pichia stipitis* could not effectively ferment carbon substrates at that temperature. *H. polymorpha* even could ferment both glucose and xylose up to 45°C.

This present study describes the use of xylose enrichment to isolate yeast from soil, leaf litter, decay wood, fruit and sediment of Enggano, and verify the important of Enggano island as microbial resources.

MATERIALS AND METHODS

Isolation of Yeasts: Yeasts were isolated from soil, leaf litter, decay wood, fruit and sediment. 0.5 g of sample was added to 4.5 mL of enrichment media containing (Yeast Nitrogen Base (DIFCO) 26.8 g/L, xylose 80 g/L, sodium propionate 2g/L, chloramphenicol 1.2 g/L and incubated for 5 days. To isolate the yeasts from the sample, 1.0 mL of sample from each enrichment media was diluted in 9 mL sterilized water and vortex-mixed. One-tenth of a milliliter of successive decimal dilutions was spread on acidified Dichloran Rose Bengal agar chloramphenicol agar (OXOID, Cat.1076012). This selective medium was used because growth of bacteria was prevented, and spreading of molds was suppressed (King *et al*., 1979).

Leaf litters 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 µL and 50 µL of these samples were plated on RBCA. For direct plating, the leaf and leaf litter were weighed and cut into small pieces of about 2 cm^2. 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 leaf litter using the ballistospore-fall method (Pringle *et al*., 2005). 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 RBCA agar germinated, and yeasts were cultivated.

Plates were incubated for 5 days at room temperature. Strain purification was done at least twice by selecting one of each type of yeast colony and streaking twice for single colonies. The plate were incubated at 27°C for 3 days. Representative colonies were picked, purified and maintained on YMA (10 g/L glucose, 5 g/L peptone, 3 g/L yeast extract, 3 g/L malt extract, and 20 g/L agar.

rDNA sequence determination. Yeast DNA template was prepared from freshly-grown cells on the PDA plate and used for colony PCR as described (Butinar *et al*., 2005). Five uL of lysed yeast cell suspension was used for PCR amplification of the partial 26SrDNA subunit with primers NL1 and NL4 (Kurztman and Robnett, 1998) 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) was used 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)(Altschul *et al*., 1990). The ITS1/5.8S/ITS2 region of selected strains was also amplified with primers ITS1 and ITS4 (Vancov and Keen, 2009) when species identifications were ambiguous.

Phylogenetic Analysis. Sequences were aligned using CLUSTAL X (Larkin *et al*., 2007). The distance matrix for the aligned sequences was calculated using the two-parameter methods of Kimura (Kimura, 1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. The neighbor-joining (NJ) method (Gascuel and Steel, 2006) was used to construct all phylogenetic trees.

Preservation of Yeast Cultures. Yeast isolates were preserved by two methods, in 20 % glycerol solution at -80°C, and by lyophilization (Bedu-addo, 2004). Yeasts were deposited in the Indonesian Culture Collection (InaCC, www.biologi.lipi.go.id) at the Research Center for Biology-Indonesian Institute of Sciences.

RESULTS

Diversity of yeasts assimilating xylose on various substrates

Taxonomically distinct yeasts were observed from the 5 samples sources (Fig. 1) collected from Enggano island. From a total of 50 samples, soil was found as a good sample source for yeast isolation. From ten samples of soil collected from 5 different sites, we isolated 90 isolates, 51 isolates were from sediment, 31 isolates were from leaf litter, 20 isolates

Table 1. Diversity of yeast species isolated from soil, leaf litter and sediment in Enggano Island, Bengkulu, Indonesia. (*Keragaman khamir yang diisolasi dari sampel tanah, seresah dan sedimen asal Pulau Enggano, Bengkulu, Indonesia*).

Taxonomic placement	Species	Origin	Organism ID
Ascomycota, *Saccharomycetes*, *Saccharomycetales*, *Lodderomyces-spathaspora* clade	*Candida tropicalis*	Soil	YEg047, YEg087, YEg148, YEg149
		Sediment	YEg263, YEg288
	Candida sojae	Soil	YEg177
	Candida albicans	Sediment	YEg299
Ascomycota, *Saccharomycetes*, *Saccharomycetales*, *Saturnispora* clade	*Candida silvae*	Soil	YEg138, YEg139
		Sediment	YEg295
Ascomycota, *Saccharomycetes*, *Saccharomycetales*, *Pichia* clade	*Candida pseudolambica*	Soil	YEg164
	Candida pseudointermedia	Sediment	YEg260
		Litter	YEg303
Ascomycota, *Saccharomycetes*, *Saccharomycetales*, *Metschnikowia*clade	*Candida insectorium*	Soil	YEg033
Ascomycota, *Saccharomycetes*, *Saccharomycetales*, *Yamadazyma* clade	*Candida boidinii*	Soil	YEg158
Ascomycota, *Saccharomycetes*, *Saccharomycetales*	*Williopsissaturnus*	Soil	YEg025, YEg155,YEg184
		Sediment	YEg267,YEg 280, YEg285,YEg289
		Litter	YEg198
	Cyberlindnera saturnus	Soil	YEg048,YEg304
		Sediment	YEg290
	Cyberlindnera mrakii	Sediment	YEg273
Ascomycota, *Saccharomycetes*, *Saccharomycetales*, Saccharomycetaceae	*Candida pseudolambica*	Soil	YEg164
	Candida yuanshanica	Litter	YEg199
		Sediment	YEg252,YEg279.YEg292
	Kluyveromyces hubeiensis	Sediment	YEg272
Basidiomycota, *Pucciniomycotina*, *Agaricostilbomycetes*, *Ruineniae* clade	*Sporobolomyces poonsookiae*	Soil	YEg186

were from decay wood and 8 isolates were from fruit. From a total of 200 isolates, a total of 76 xylose assimilating yeast were isolated and identified during this study. They belong to two lineages of Fungi, Saccharomycotina (15 species), and Puciniomycotina (1 species). Representative colonies morphological of isolated yeasts were shown in Figure 2.

As shown in Table 1, most the isolates were ascomycetous yeast and one isolate was basidiomycetous yeast. Ascomycetous yeasts isolated in this study were classified into one subphylum Saccharomycotina in which most frequently isolated from soil and sediment. Whereas strains belong to basidiomycetous yeast were isolated from soil only.

The most frequently isolated species was *Williopsis saturnus* which found in soil, sediment and leaf litter, respectively for a total of 9 individual isolations. The next most frequently isolated species was *Candida tropicalis*, with 6 individual isolations, although it was more common in soil samples. This was followed closely by *Cyberlindnera saturnus*, with 4 isolations.

Yeasts were also isolated from leaf litter, which were identified as *Candida yuanshanica* and *Candida intermedia*. The common yeast assimilating xylose species on soil were: *C. insectorum*, *C. silvae*, *C. boidinii*, *C. pseudolambica*, *C. tropicalis*, *C. sojae*, *W. saturnus*, *Cyberlindnera saturnus*, and one new species identified as *Candida* sp in Saccharomycotina, Ascomycota; and

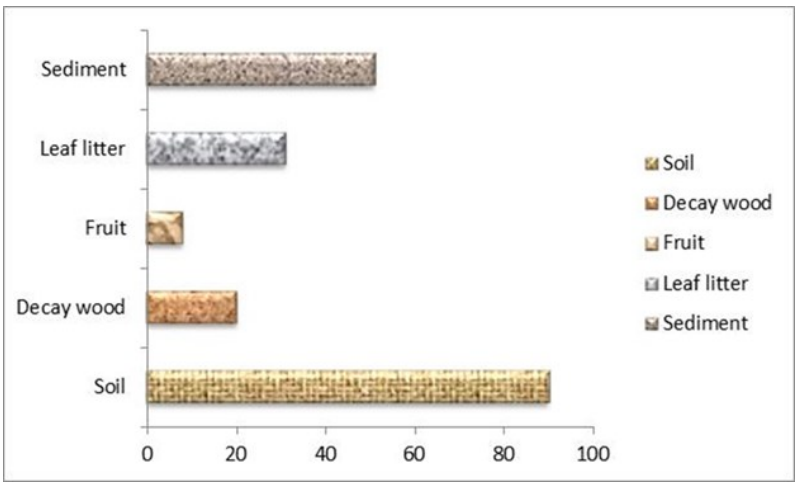

Figure 1. Number of xylose assimilating yeast isolates obtained from the island of Enggano. (*Jumlah isolat Khamir yang mempunyai kemampuan asimilasi xilose yang diisolasi dari Pulau Enggano*).

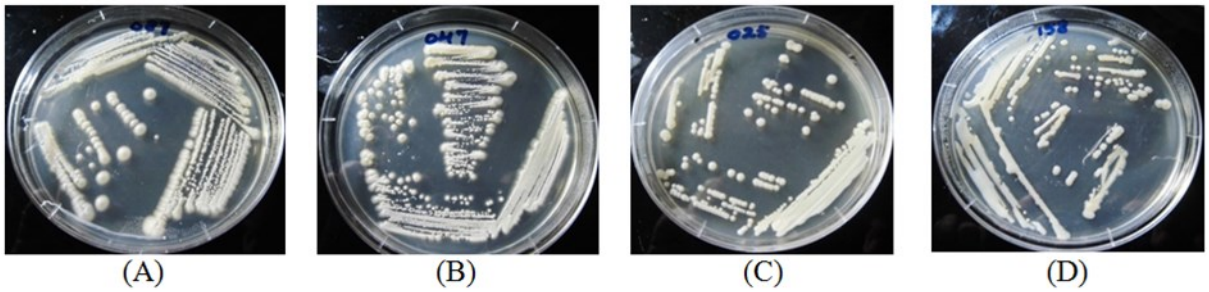

Figure 2. Representative colony morphology:(A) *Candida insectorum*, (B) *Candida tropicalis*, (C) *Williopsis saturnus* and (D) *Candida bodinii* isolated from Island of Enggano. (*Morfologi koloni Khamir asal Pulau Enggano*).

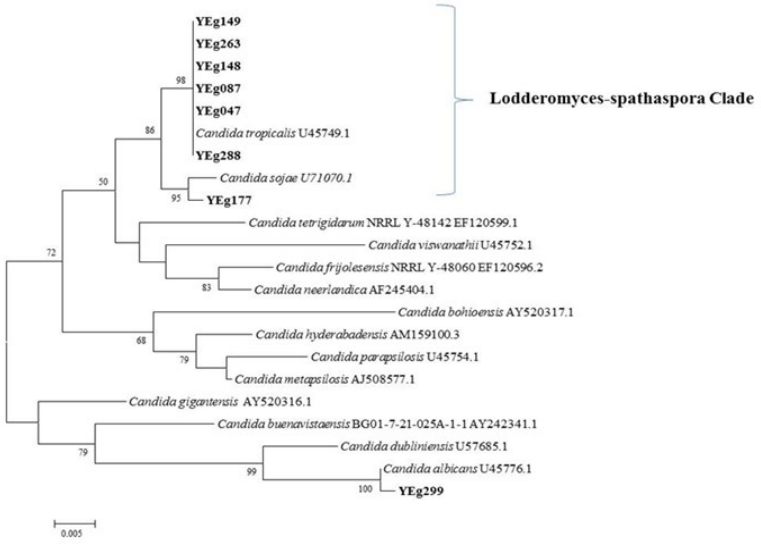

Figure 3. Phylogram of yeast assimilating xylose isolated from Enggano Island, and their position within the Lodderomyces-spathaspora clade based on D1/D2 rDNA sequences. (*Filogram hubungan Khamir asal Pulau Enggano yang mempunyai potensi asimilasi xilose grup Lodderomyces-spathaspora berdasarkan pemetaan sekuen daerah D1/D2 rDNA*).

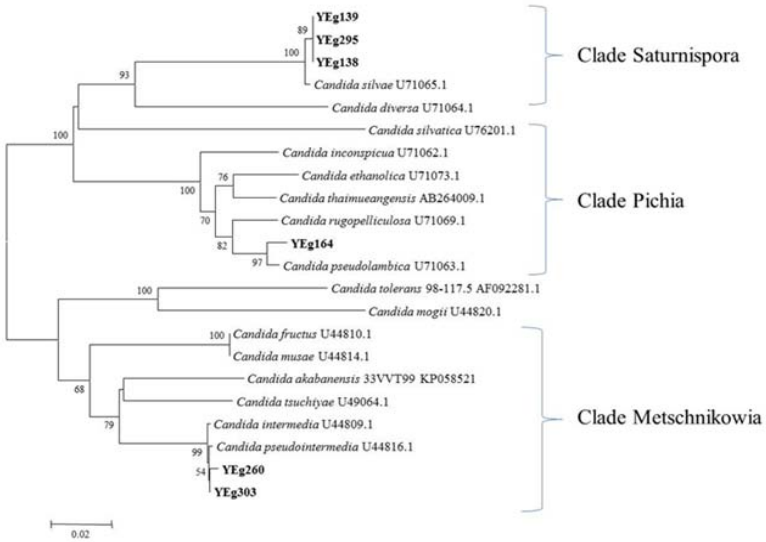

Figure 4. Phylogram of yeast isolates, and their position within Clade *Saturnispora*, *Pichia*, *Metschnikowia* in the class *Saccharomycetes* based on D1/D2 of LSU rDNA sequences. (*Filologram isolat khamir dan posisinya pada grup Saturnispora, Pichia, Metschnikowia dalam kelas Saccharomycetes berdasarkan pemetaan sekuen daerah D1/D2 rDNA*).

Sporobolomyces poonsookiae in Pucciniomycotina, Basidiomycota. The species found at relatively high frequencies from sediment were: *C. silvae*, *C. yuanshanica*, *C. tropicalis*, *W. saturnus*, *Cy. saturnus* and *Cy. mrakii*. Finally, *Kluyveromyces hubeiensis* was the only species collected only once.

Phylogenetic position of isolates

Seventy six strains of yeast were isolated and identified from soil, leaf litter, decay wood, fruit and sediment in Enggano Island, Indonesia. Molecular identification revealed that the yeast strains are taxonomically diverse, belonging to the phyla Ascomycota and Basidiomycota. Yeasts within the

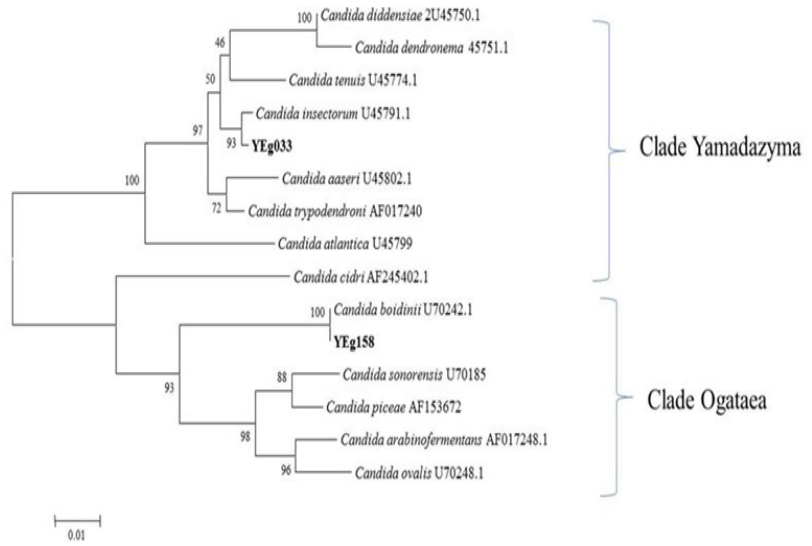

Figure 5. The phylogram of yeast isolated from Enggano island, within the Subphylum Saccharomycotina as inferred from D1/D2 ofLSU rDNA sequences. (Phylogram *Khamir asal Pulau Enggano pada subfilum Saccharomycotina berdasarkan pemetaan sekuen daerah D1/D2 Rdna*).

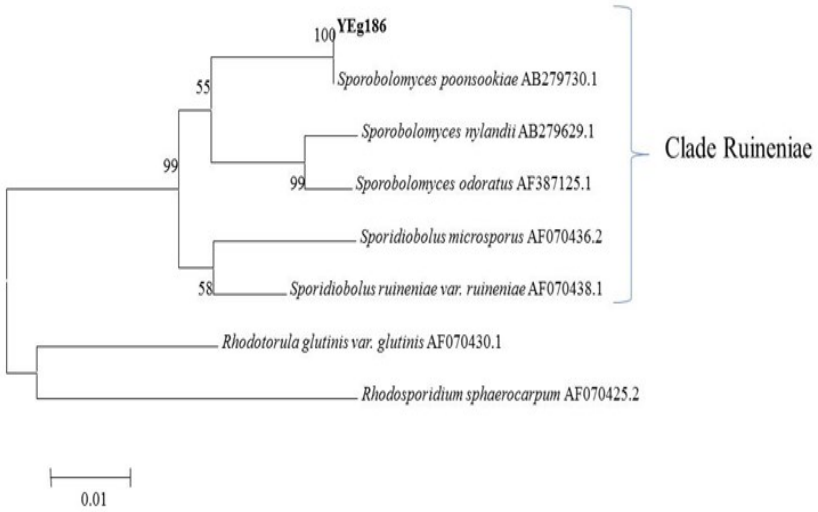

Figure 6. Neighbor-joining tree constructed using the D1/ D2 region of the nLSU rDNA sequences of Sporobolomyces species and its related species. (*Kontruksi pohon filogenetik berdasarkan pemetaan sekuen daerah D1/D2 jenis Sporobolomyces dan jenis kerabat*).

phylum Ascomycota are in the more commonly encountered subphylum Ascomycotina. Yeasts within the phylum Basidiomycota include those in subphylum Pucciniomycotina. Based on sequence analyses of D1/D2 domain of the LSU rDNA, these 76 strains were tentatively classified into 7 clades (Figure 3 – Figure 6). The most frequent yeast species belong to genus *Candida* with consist of 11 species. *Cyberlindnera* was the predominant genus with 2 species isolations. *Williopsis* was the next most common, followed by *Sporobolomyces* and *Kluyveromyces* with 1 species isolation for each genus.

Candida species were placed in the family Wickerhamomycetaceae, clades *Lodderomyces, Saturnispora*; family Metschnikowiaceae clade

Metschnikowia; family *Pichiaceae* clade *Pichia*; and family *incertaesedis* clade Ogataea; family Debaryomycetacea clade *Yamadazyma*. *Kluyveromyces* species was placed in the family *Saccharomycetaceae* clade *Kluyveromyces*. Basidiomycota species included those belonging to class Agaricostilbomycetes, order Spiculogloeales, clade Sporobolomyces.

DISCUSSION

Little information was previously available about yeasts of Enggano, Indonesia. One of islands that makes up this richly biodiverse and biogeographically significant region (Jakl, 2008). We found a broad taxonomic diversity of yeast species from this exploratory survey. The isolated strains mostly belong to Ascomyceteous yeast, of whose most of the xylose fermenting yeast resides under this group (Kurtzman and Robnett, 1998; Urbina*et al*., 2013). However, numerous potentially novel species were obtained from island of Enggano. Novel strains of *Candida* sp. originated from soil were isolated, expanding the known geographic and habitat range of these known species.

Sample sites or microbial sources affected distribution and taxonomic composition of yeasts in Island of Enggano. Soil and sediment were best sources for yeast (Figure 1), followed by leaf litter and decay wood. Less yeast was obtained from fruit. *Candida* was ubiquitous yeast that can be isolated from all sample sources. *Candida* is fastidious yeast as shown by *Candida famata* and *Rhodothorula rubra* which were dominant species cultivated from sediment of lagoon (Bogusławska-Was and Dabrowski 2001), which implies that *Candida* is widely distributed from terrestrial to aquatic ecosystem. Some yeast species such as *Cryptococcus albidus*, *Cry. laurentii, Rhodothorula glutinis*, *R. colostri*, and *Debaryomyce shansenii*, however, were commonly found in all litter types. But, other yeasts were restricted to a specific type of litter, implying that type of litters affect yeast diversity (Sampaio *et al*., 2004).

The most frequently isolated yeast genus from Island of Enggano was *Candida.* This well-known genus is a polyphyletic, 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.* Due to its taxonomic diversity, it is not surprising that *Candida* is ecologically diverse also, occupying niches including human infections, soil, insect frass, fruit, and many other habitats. However, clades of *Candida* are currently being reassigned to new genera to more accurately reflect their taxonomic placement. Therefore, many yeasts classified as *Candida* in this study may soon be classified in other genera.

Microbes such as yeasts and bacteria are essential for xylose fermentation (Kuyper *et al*., 2005). The larger cell size, thicker cell wall, better growth at lower pH, less stringer nutrient requirement, greater resistance to contamination faster, is advantage of yeast over bacteria for commercial fermentation (Morais *et al*., 2013). For biofuel development, we obtained 76 isolates yeasts that are able to utilize xylose. Xylose assimilation is important character of yeasts that used for economical biofuel development (Kuyper *et al*., 2003). Soil and sediment were the best sources for xylose assimilating yeast (Figure 1).

Distribution of yeast in soil is strongly affected by physicochemical soil properties. The negative effects of soil pH on the yeast numbers observed in low nutrient soil (Vreulink *et al*., 2007). Using general regression models, they demonstrated that soil pH and copper concentration were the 2 variables that correlated best with soil yeast counts in the soils. However, soil moisture content was found to be the environmental factor with the most impact on heterotrophic microbes. Divalent cation availability might also impact on the size of both yeast and prokaryote populations in the soils.

Yeast performed pentose phosphate pathways for xylose metabolism which produce D-ribose for nucleic acid biosynthesis, D-erythrose 4-phosphate for the synthesis of aromatic amino acids and NADPH for anabolic reactions. The final metabolites produced are acetyl-Co-A, and acetaldehyde. The later metabolite is then reduced into ethanol in the presence of NADPH (Zhang and Geng, 2012).

Many of the species isolated in this study have potential applications. Candida was ubiquitous which include *C. insectorum*, *C. tropicalis*, *C. boidinii*, *C. pseudolambica, C. yuanshanica*, and *C. silvae*. Other

important species were *Cyberlindnera saturnus*, *Williopsis saturnus* and *Sporobolomyces poonsookiae*.

Important applications using *Candida* species include agent for bioremediation, *C. catenulate* (Trodler *et al.*, 2008), and biofuel *C. tropicalis* (Stringini *et al.*, 2008). *Candida tropicalis* isolated repeatedly in this study is a well-known species having wide distribution and having high xylose transport capacity. *Sporobolomyces poonsookiae* shows promise for bio control of the fungal plant pathogen *Botrytis cinerea* (Trillas *et al.*, 2006).

Plant biomass is important sources of lignocellulose. The use of plant biomass for biofuel production will require efficient utilization of the sugars in lignocellulose, primarily glucose and xylose. However, the most well-known strains of *Saccharomyces cerevisiae* presently used in bioethanol production ferment glucose but not xylose. To overcome these bottlenecks, Kim *et al.*, 2013 constructed yeasts that co- ferment mixtures of xylose and cellobiose. They proposed hydrolysis of cellobiose occurs inside yeast cells through the action of an intracellular β-glucosidase following import by a high-affinity cellodextrin transporter. Owing to intracellular hydrolysis of cellobiose will result in minimizing glucose repression of xylose fermentation. This will allow co-consumption of cellobiose and xylose (Kim *et al.*, 2013). The resulting yeast strains, co-fermented cellobiose and xylose simultaneously and exhibited improved ethanol yield when compared to fermentation with either cellobiose or xylose as sole carbon sources. They obtained better yields and productivities from co-fermentation. The successful integration of cellobiose and xylose fermentation pathways in yeast is a critical step towards enabling economic biofuel production (Kuyper *et al.*, 2005).

Isolation and characterization of xylose fermenting yeast is interest of many scientists (Morais *et al.*, 2013). Well known xylose fomenting yeast are *Hansenula polymorpha,* and *Pichia stipitis*. Other important wild strains are *Spathaspora passalidarum*, *Scheffersomyces stipitis* which were isolated from Brazil (Cadete *et al.*, 2012). When grown in D-xylose (50 g/L) culture medium, *S. passalidarum* strains produced the highest ethanol yields (0.37 g/g) and productivities (0.62 g/L. h to 0.75 g/L. h). However, *C. amazonensis* exhibited a virtually complete D-xylose consumption and the highest xylitol yields (0.55 g/g to 0.59 g/g), with concentrations up to 25.2 g/L.

This study supports other studies that concluded that xylose assimilating yeast can be isolated from various sources (Cadete *et al.*, 2012), and island of Enggano is biodiversity hot spot area for xylose assimilating yeast.

CONCLUSION

Seventy six isolates of xylose assimilating yeast were obtained from Enggano Island, Bengkulu. They mostly belong to *Candida* which includes *Candida insectorum*, *C. tropicalis*, *C. boidinii*, *C. pseudolambica, C. yuanshanica*, *C. silvae*, and other isolates namely *Cyberlindnera saturnus*, *Williopsis saturnus* and *Sporobolomyces poonsookiae*. One strain, isolated from soil, *Candida* sp. based on analyses of gene sequence from D1/D2 domain could be assigned as a candidate of novel species. This study confirms that Island of Enggano is important biodiversity hot spot, and as genetic resources for taxonomic study and bioprospecting.

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Pedoman Penulisan Naskah Berita Biologi

Berita Biologi adalah jurnal yang menerbitkan artikel kemajuan penelitian di bidang biologi dan ilmu-ilmu terkait di Indonesia. Berita Biologi memuat karya tulis ilmiah asli berupa makalah hasil penelitian, komunikasi pendek dan tinjauan kembali yang belum pernah diterbitkan atau tidak sedang dikirim ke media lain. Masalah yang diliput, diharuskan menampilkan aspek atau informasi baru.

Tipe naskah

1. **Makalah lengkap hasil penelitian (*original paper*)**
   Naskah merupakan hasil penelitian sendiri yang mengangkat topik yang *up-todate*. Tidak lebih dari 15 halaman termasuk tabel dan gambar. Pencantuman lampiran seperlunya, namun redaksi berhak mengurangi atau meniadakan lampiran.
2. **Komunikasi pendek (*short communication*)**
   Komuniasi pendek merupakan makalah hasil penelitian yang ingin dipublikasikan secara cepat karena hasil termuan yang menarik, spesifik dan baru, agar dapat segera diketahui oleh umum. Artikel yang ditulis tidak lebih dari 10 halaman. Hasil dan pembahasan boleh digabung.
3. **Tinjauan kembali (*review*)**
   Tinjauan kembali merupakan rangkuman tinjauan ilmiah yang sistematis-kritis secara ringkas namun mendalam terhadap topik penelitian tertentu. Hal yang ditinjau meliputi segala sesuatu yang relevan terhadap topik tinjauan yang memberikan gambaran '*state of the art*', meliputi temuan awal, kemajuan hingga issue terkini, termasuk perdebatan dan kesenjangan yang ada dalam topik yang dibahas. Tinjauan ulang ini harus merangkum minimal 30 artikel.

Struktur naskah

1. **Bahasa**
   Bahasa yang digunakan adalah bahasa Indonesia atau Inggris yang baik dan benar.
2. **Judul**
   Judul harus singkat, jelas dan mencerminkan isi naskah diikuti oleh nama dan alamat surat menyurat penulis. Nama penulis untuk korespondensi diberi tanda amplop cetak atas (*superscript*).
3. **Abstrak**
   Abstrak dibuat dalam dua bahasa, bahasa Indonesia dan Inggris. Abstrak memuat secara singkat tentang latar belakang, tujuan, metode, hasil yang signifikan, kesimpulan dan implikasi hasil penelitian. Abstrak berisi maksimum 200 kata, spasi tunggal. Di bawah abstrak dicantumkan kata kunci yang terdiri atas maksimum enam kata, dimana kata pertama adalah yang terpenting. Abstrak dalam bahasa Inggris merupakan terjemahan dari bahasa Indonesia. Editor berhak untuk mengedit abstrak demi alasan kejelasan isi abstrak.
4. **Pendahuluan**
   Pendahuluan berisi latar belakang, permasalahan dan tujuan penelitian. Sebutkan juga studi terdahulu yang pernah dilakukan.
5. **Bahan dan cara kerja**
   Pada bagian ini boleh dibuat sub-judul yang sesuai dengan tahapan penelitian. Metoda harus dipaparkan dengan jelas sesuai dengan standar topik penelitian dan dapat diulang oleh peneliti lain. Apabila metoda yang digunakan adalah metoda yang sudah baku cukup ditulis sitasi dan apabila ada modifikasi harus dituliskan dengan jelas bagian mana dan apa yang dimodifikasi.
6. **Hasil**
   Sebutkan hasil-hasil utama yang diperoleh berdasarkan metoda yang digunakan. Apabila ingin mengacu pada tabel/grafik/diagram atau gambar uraikan hasil yang terpenting dan jangan menggunakan kalimat 'Lihat Tabel 1'. Apabila menggunakan nilai rata-rata harus menyebutkan standar deviasi.
7. **Pembahasan**
   Jangan mengulang isi hasil. Pembahasan mengungkap alasan didapatkannya hasil dan apa arti atau makna dari hasil yang didapat tersebut. Bila memungkinkan, bandingkan hasil penelitian ini dengan membuat perbandingan dengan studi terdahulu (bila ada).
8. **Kesimpulan**
   Menyimpulkan hasil penelitian, sesuai dengan tujuan penelitian, dan penelitian berikut yang bisa dilakukan.
9. **Ucapan terima kasih**
10. **Daftar pustaka**
    Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses peer review. Apabila harus menyitir dari "Laporan" atau "komunikasi personal" dituliskan *'unpublished'* dan tidak perlu ditampilkan di daftar pustaka. Daftar pustaka harus berisi informasi yang *up to date* yang sebagian besar berasal dari *original papers*. Penulisan terbitan berkala ilmiah (nama jurnal) tidak disingkat.

Format naskah

1. Naskah diketik dengan menggunakan program Word Processor, huruf New Times Roman ukuran 12, spasi ganda kecuali Abstrak. Batas kiri -kanan atas-bawah masing-masing 2,5 cm. Maksimum isi naskah 15 halaman termasuk ilustrasi dan tabel.
2. Penulisan bilangan pecahan dengan koma mengikuti bahasa yang ditulis menggunakan dua angka desimal di belakang koma. Apabila menggunakan bahasa Indonesia, angka desimal menggunakan koma (,) dan titik (.) bila menggunakan bahasa Inggris. Contoh: Panjang buku adalah 2,5cm. Lenght of the book is 2.5 cm. Penulisan angka 1-9 ditulis dalam kata kecuali bila bilangan satuan ukur, sedangkan angka 10 dan seterusnya ditulis dengan angka. Contoh lima orang siswa, panjang buku 5 cm.
3. Penulisan satuan mengikuti aturan *international system of units*.
4. Nama takson dan kategori taksonomi merujuk kepada aturan standar termasuk yang diakui. Untuk tumbuhan *International Code of Botanical Nomenclature* (ICBN), untuk hewan *International Code of Zoological Nomenclature* (ICZN), untuk jamur *International Code of Nomenclature for Algae, Fungi and Plant* (ICFAFP), *International Code of Nomenclature of Bacteria* (ICNB), dan untuk organisme yang lain merujuk pada kesepakatan Internasional. Penulisan nama takson lengkap dengan nama author hanya dilakukan pada bagian deskripsi takson, misalnya pada naskah taksonomi. Sedangkan penulisan nama takson untuk bidang lainnya tidak perlu menggunakan nama author.
5. Tata nama di bidang genetika dan kimia merujuk kepada aturan baku terbaru yang berlaku.
6. Ilustrasi dapat berupa foto (hitam putih atau berwarna) atau gambar tangan (*line drawing*).
7. Tabel
   Tabel diberi judul yang singkat dan jelas, spasi tunggal dalam bahasa Indonesia dan Inggris, sehingga Tabel dapat berdiri sendiri. Tabel diberi nomor urut sesuai dengan keterangan dalam teks. Keterangan Tabel diletakkan di bawah Tabel. Tabel tidak dibuat tertutup dengan garis vertikal, hanya menggunakan garis horisontal yang memisahkan judul dan batas bawah. Paragraf pada isi tabel dibuat satu spasi.
8. Gambar
   Gambar bisa berupa foto, grafik, diagram dan peta. Judul ditulis secara singkat dan jelas, spasi tunggal. Keterangan yang menyertai gambar harus dapat berdiri sendiri, ditulis dalam bahasa Indonesia dan Inggris. Gambar dikirim dalam bentuk .jpeg dengan resolusi minimal 300 dpi.
9. Daftar Pustaka
   Sitasi dalam naskah adalah nama penulis dan tahun. Bila penulis lebih dari satu menggunakan kata 'dan' atau *et al.* Contoh: (Kramer, 1983), (Hamzah dan Yusuf, 1995), (Premachandra *et al.,* 1992). Bila naskah ditulis dalam bahasa Inggris yang menggunakan sitasi 2 orang penulis

maka digunakan kata 'and'. Contoh: (Hamzah and Yusuf, 1995).

a. Jurnal
   Nama jurnal ditulis lengkap.
   Premachandra GS, H Saneko, K Fujita and S Ogata. 1992. Leaf Water Relations, Osmotic Adjustment, Cell Membrane Stability, Epi-cutilar Wax Load and Growth as Affected by Increasing Water Deficits in Sorghum. *Journal of Experimental Botany* **43,** 1559-1576.
b. Buku
   Kramer PJ. 1983. *Plant Water Relationship*, 76. Edisi ke-(bila ada). Academic, New York.
c. Prosiding atau hasil Simposium/Seminar/Lokakarya.
   Hamzah MS dan SA Yusuf. 1995. Pengamatan Beberapa Aspek Biologi Sotong Buluh *(Sepioteuthis lessoniana)* di Sekitar Perairan Pantai Wokam Bagian Barat, Kepulauan Aru, Maluku Tenggara. *Prosiding Seminar Nasional Biologi XI,* Ujung Pandang 20-21 Juli 1993. M Hasan, A Mattimu, JG Nelwan dan M Litaay (Penyunting), 769-777. Perhimpunan Biologi Indonesia.
d. Makalah sebagai bagian dari buku
   Leegood RC and DA Walker. 1993. Chloroplast and Protoplast. In: *Photosynthesis and Production in a Changing Environment.* DO Hall, JMO Scurlock, HR Bohlar Nordenkampf, RC Leegood and SP Long (Eds), 268-282. Champman and Hall. London.
e. Thesis dan skripsi.
   Keim AP. 2011. Monograph of the genus *Orania* Zipp. (Arecaceae; Oraniinae). University of Reading, Reading. [PhD. Thesis].
f. Artikel online.
   Artikel yang diunduh secara online mengikuti format yang berlaku misalnya untuk jurnal, buku atau thesis, serta dituliskan alamat situs sumber dan waktu mengunduh. Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses *peer review* atau artikel dari laman web yang tidak bisa dipertangung jawabkan kebenarannya seperti wikipedia.
   Forest Watch Indonesia[FWI]. 2009. Potret keadaan hutan Indonesia periode 2000-2009. *http://www.fwi.or.id*. (Diunduh 7 Desember 2012).

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Penelitian yang melibatkan hewan

Untuk setiap penelitian yang melibatkan hewan sebagai obyek penelitian, maka setiap naskah yang diajukan wajib disertai dengan '*ethical clearance approval*' terkait *animal welfare* yang dikeluarkan oleh badan atau pihak berwenang.

Lembar ilustrasi sampul

Gambar ilustrasi yang terdapat di sampul jurnal Berita Biologi berasal dari salah satu naskah. Oleh karena itu setiap naskah yang ada ilustrasi harap mengirimkan ilustrasi dengan kualitas gambar yang baik disertai keterangan singkat ilustrasi dan nama pembuat ilustrasi.

Proofs

Naskah *proofs* akan dikirim ke author dan diwajibkan membaca dan memeriksa kembali isi naskah dengan teliti. Naskah proofs harus dikirim kembali ke redaksi dalam waktu tiga hari kerja.

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