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Isolation and Identification of Ethanol and Glucose Tolerance Yeasts Strain from *Tacca leontopetaloides*

GEMILANG LARA UTAMA*, WAHYU KRISTIAN SUGANDI, ELAZMANAWATI LEMBONG, AND EDY SURYADI

Faculty of Agro-Industrial Technology, Universitas Padjadjaran
Jalan Raya Bandung – Sumedang KM 21 Jatinangor UBR 40600, West Java, Indonesia.

The research aimed to isolate and characterize indigenous yeast strain from *Tacca leontopetaloides* with respect to the ethanol and glucose tolerance ability. Research done experimentally and the data analyzed descriptively. Yeasts isolated from 1 g of *Tacca leontopetaloides* were grown at modified Potato Dextrose Agar/PDA (Oxoid Ltd.) with 3% Yeasts Extract/YE (Kraft Foods) and 10 ppm amoxicillin addition. Yeasts-like colony was tested in the ability to tolerate ethanol and glucose contents by growing on modified Nutrient Broth/NB (Oxoid Ltd.) with 3% YE and 10 ppm amoxicillin then added with glucose monohydrate (10%, 20%, 30%) or ethanol (10%, 20%, 30%) and incubated for 72 h at ambient (23-28 °C). Optical density (OD) was read for UV absorbance at 600 nm using UV-Vis spectrophotometer every 24 h until 72 h. The strain of best isolate with the ability to tolerate high ethanol and glucose contents were identified by the sequence analysis of ITS (Internal Transcribed Spacer) region using primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The sequencing was performed at Macrogen Inc. (Seoul, South Korea), and the sequences was compared with the GenBank database using BLAST (Basic Local Alignment Search Tools) algorithm (http//:www.ncbi.nlm.nih.gov/BLAST/). Results showed there are four yeasts-like isolate and the TK1 isolate showed the best ethanol tolerance ability with highest OD at 30% ethanol concentration (0.486) and the highest OD at 30% glucose concentration (1.732). The species identification of TK1 isolate was identical with *Candida quercitrusa* JHSb.

Key words: ethanol tolerance, glucose tolerance, indigenous yeasts, *Tacca leontopetaloides*

Penelitian bertujuan untuk mengisolasi dan mengkarakterisasi jenis khamir *indigenous* asal *Tacca leontopetaloides* dengan ketahanannya terhadap etanol dan glukosa. Penelitian dilakukan secara eksperimental dan data dianalisis secara deskriptif. Khamir diisolasi dari 1g *Tacca leontopetaloides* kemudian ditumbuhkan pada *Potato Dextrose Agar*/PDA (Oxoid Ltd.) dengan penambahan 3% *Yeasts Extract*/YE (*Kraft Foods*) dan 10 ppm amoxicillin. Koloni menyerupai khamir diuji kemampuannya dalam ketahanan terhadap etanol dan glukosa dengan ditumbuhkan pada *Nutrient Broth*/NB (Oxoid Ltd.) dengan 3% YE dan 10 ppm amoxicillin yang ditambahkan etanol (10%, 20%, 30%) atau glukosa monohidrat (10%, 20%, 30%) dan kemudian diinkubasi selama 72 jam pada suhu ruang (23-28°C). Kerapatan optik dibaca pada panjang gelombang 600 nm menggunakan spektrofotometer UV-Vis setiap 24 jam sampai 72 jam. *Strain* isolat terbaik dengan ketahanan terhadap kadar etanol dan glukosa yang tinggi diidentifikasi dengan analisis sekuen ITS (*Internal Transcribed Spacer*) *region* dengan menggunakan *primer* ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) dan ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) dengan bantuan Macrogen Inc. (Seoul, Korea Selatan), sekuen kemudian dibandingkan dengan *database* GenBank dan algoritma *Basic Local Alignment Search Tools* / BLAST (http//:www.ncbi.nlm.nih.gov/BLAST/). Hasil menunjukkan bahwa terdapat 4 isolat menyerupai khamir dan isolat TK1 menunjukkan ketahanan terhadap etanol dan glukosa terbaik dengan kerapatan optik tertinggi pada konsentrasi etanol 30% mencapai 0,486 dan and kerapatan optik tertinggi pada konsentrasi glukosa 30% mencapai 1,732. Identifikasi species khamir menunjukkan bahwa isolat TK1 identik dengan *Candida quercitrusa* JHSb.

Kata kunci: ketahanan etanol, ketahanan glukosa, khamir Indigenous, *Tacca leontopetaloides*

Taka (*Tacca leontopetaloides*) is one of the starch sources found growing wild in coastal areas of Indonesia. Despite of the high content of starch, which reaches 66.65% consisting of 22.7% amylose and 43.88% amylopectin (Aatjin *et al.* 2013), Taka tuber cannot be directly consumed because it contained bitter compound of Taccaline. Therefore, the utilization of Taka tuber is still limited However, with certain treatment some people in coastal area could consume Taka as substitutes for rice, and Taka flour is used to make various types of cakes.

On the other side, the low utilization of Taka tuber for human consumption make it available to be used as

*Corresponding author: Phone: +62-81220272894, Email: g.l.utama@unpad.ac.id

a material for producing bioethanol by the help of microorganisms. Starch contained by Taka potential to produce oligosaccharides through enzymatic hydrolysis process. Further, the oligosaccharides can be utilized by microorganisms such as yeast in producing renewable energy sources such as bioethanol (Thatoi *et al.* 2014).

However, the use of yeasts in converting Taka starch is very risky to find obstacles, given the high content of phytochemicals contained in tubers of Taka. The number of phytochemical components such as flavonoids in fresh tuber of 3.15%, while in the planted tubers of 3.58% (Ukpabi *et al.* 2009). Taka tubers also contain oxalic compounds, cyanides, phytates, alkaloids, tannins and saponins that can act as toxic compound (Ndouyang *et al.* 2014; Ndouyang *et al.* 2015). In addition to the phytocemicals, stress during fermentation can also formed and suppress the growth of yeasts and efficieny of bioethanol conversion. Stress such as high ethanol and sugar concentration are often cause the inefficacy of bioethanol conversion (Alexandre and Charpentier 1998; Aguilar-Uscanga *et al.* 2011).

Utilization of Taka indigenous yeasts role as an agent in the conversion of bioethanol can be one way to overcome the obstacle. Indigenous yeasts could adapt the extreme conditions of the entire substrate and perform good bioethanol conversion. Indigenous yeast has been widely isolated from biomass to be identified for its ability to convert bioethanol. *Candida krusei* has been found as indigenous yeasts isolated from vegetable waste that has the ability to tolerate stress and converting bioethanol up to 11.2% from cellulose-based biomass (Utama *et al.* 2017).

The utilization of highly stress-tolerant indigenous yeasts of Taka to produce bioethanol from Taka tuber can be considered beneficial. Therefore, this research aims to determine indigenous yeast strain from *Tacca leontopetaloides* with the tolerance towards ethanol and glucose contents.

MATERIALS AND METHODS

Indigenous yeasts isolation has been done by culturing 1 g of *Tacca leontopetaloides* at modified Potato Dextrose Agar (PDA) (Oxoid Ltd.) with the addition of 3% Yeasts Extract (Kraft Inc.) and 10 ppm amoxicillin, by pour plate method (modification of Utama *et al.* 2016). The Taka sample used was a mix of various part of Taka, including seeds and tuber that has been cleaned up with water. To determine the tolerance towards ethanol and glucose contents, yeasts-like colony grown on modified Nutrient Broth (Oxoid Ltd.) with addition of 3% YE (Kraft Inc.) and 10 ppm amoxicillin then added with ethanol (10%, 20%, 30%) or glucose monohydrate (10%, 20%, 30%). Samples incubated for 72h at room temperature (23-28 °C) and every 24 h the Optical density (OD) was read for UV absorbance at 600 nm using UV-Vis spectrophotometer (Fakhruddin *et al.* 2013). To determine the strain sequence analysis of ITS region performed Macrogen Inc. (Seoul, South Korea) using primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and the percentage of fragment similarity was calculated by BLAST program (http//:www.ncbi.nlm.nih.gov/BLAST/) (Maxwell *et al.* 2016).

RESULTS

Indigenous Yeasts Ability in Tolerate Various Ethanol Concentration. The results showed that four isolates had grown in the modified medium and suspected as yeast, then the isolates were tested for their ability to tolerate high levels of ethanol by observing the absorbance at 600 nm wavelength every 24 h for 72 h (Fig 1). The results showed that as the alcohol concentration increases, the yeast density tends to decrease. TK1 yeast isolate showed the best tolerance among the four yeast isolates with tolerance ability at the highest concentration of ethanol (30%) indicated by absorbance up to 0.485 at 48 hours. Other isolates such as TK2 and TK4 are isolates that have low tolerance to alcohol. At all concentrations, the absorbance from the 48th hour to the 72nd hour decreased, it means that yeast growth has been distrubed by alcohol.

Indigenous Yeasts Ability in Tolerate Various Glucose Concentration. The four yeast-like isolates have the ability to survive in substrates with glucose concentrations up to 30% indicated by increasing absorbance up to 48 h (1.732) (Fig 2). The higher glucose concentration, showed the less absorbance or lower yeast growth. However, only TK1 isolates showed a consistent response to increased amounts of glucose. TK3 and TK4 yeast isolates showed a low tolerance of glucose at all concentration.

Indigenous Yeast Strain Identification. The results of BLAST contiguous fragment with ITS1 primer on Fig 3 has shown 578 of 583 DNA sequence (99%) was similar with *Candida natalensis*. The phylogenetic tree on Fig 4 also showed that the TK1

isolate has similarity with *Candida natalensis* (KF728775.1). However, the results of ITS 4 primer on the BLAST contiguous fragment (Fig 5) have shown 564 bp of DNA sequence (100%) had similarity with *Candida quercitrusa* and the phylogenetic tree on the Fig 6 showed also that the isolate of TK1 was closed to the group of *Candida quercitrusa* (DQ665264.1). The results (Table 1) can conclude that TK1 isolate has 100% similarity with *Candidda quercitrusa* JHSb.

DISCUSSION

Indigenous Yeasts Ability in Tolerate Various Ethanol Concentration. Alcohol is harmful to yeasts because it can play a role in inhibiting yeast growth as the concentration increases in the substrate (Ali and Khan 2014). The presence of alcohol can destroy mitochondrial DNA in yeast cells and result in inactivation of hexocinase and dehydrogenase

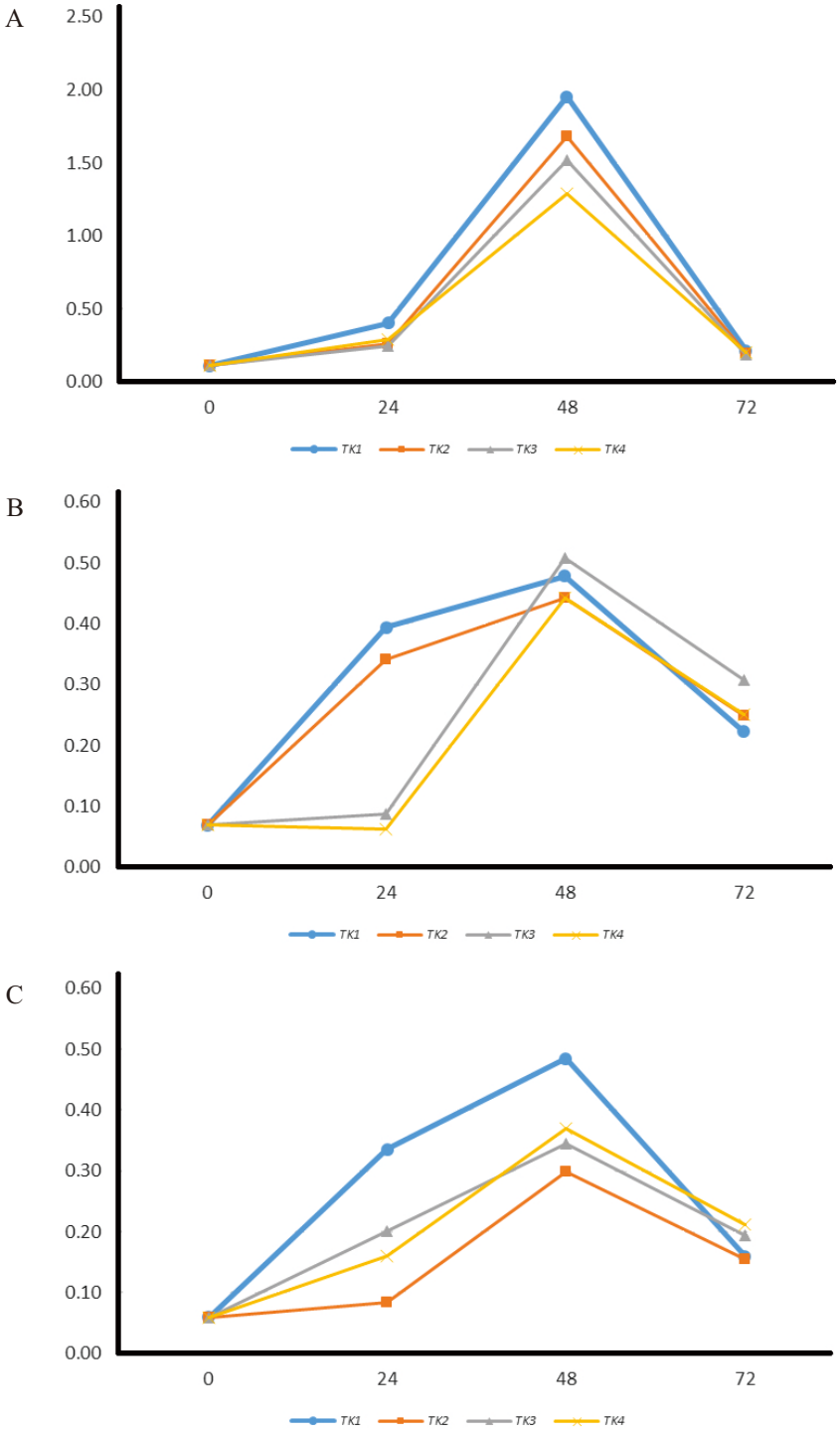

Fig 1 Yeast-like isolate tolerance towards (A) 10% Ethanol; (B) 20% Ethanol; (C) 30% Ethanol.

enzymes (Ibeas and Jimenez 1997). It causes disturbance and may decrease growth rate, fermentation rate and cell viability of yeast.

Some types of yeasts, have the ability to survive in a substrate with an alcohol concentration of up to 12% and some can survive up to a 14% alcohol concentration (Tikka *et al.* 2013). The ability to defend themselves from stress caused by high levels of alcohol, is related to the fatty acid composition possessed by the yeast cell wall (You, Rosenfield and Knipple 2003). Moneke *et al.* (2008) states that yeasts isolated from soils have the ability to survive in concentrations of 20% alcohol

Indigenous Yeasts Ability in Tolerate Various Glucose Concentration. High glucose concentration is one factor that can inhibit the growth of yeast. Charoenchai, Fleet and Henschke (1998) and D'Amato *et al.* (2006) reported that the sugar concentration of

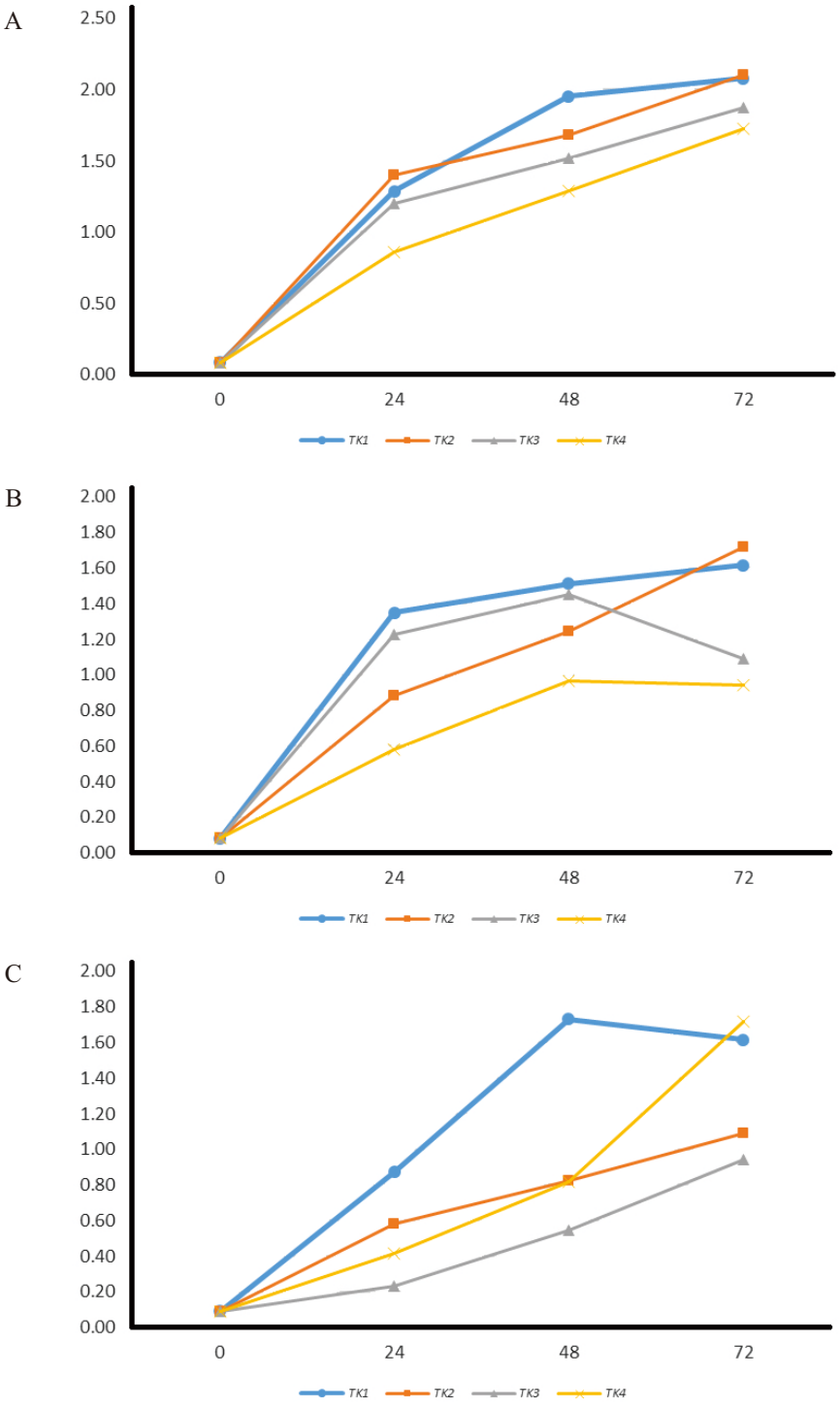

Fig 2 Yeast-like isolate tolerance towards (A) 10% Glucose; (B) 20% Glucose; (C) 30% Glucose.

A

Range 1: 1 to 580 GenBank Graphics ▼ Next Match ▲ Previous Match

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Query  72   GAAAACATTGCTTTGGTCTGGCGCAAGTTGGGCCAAAGATTTATTAAACTTCAATATTTT  131
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Query  252  AATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGG  311
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Query  312  TATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAATCTTCGGATTTGGTAT  371
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Sbjct  299  TATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAATCTTCGGATTTGGTAT  358

Query  372  TGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAAAGTATTGGCAAGAGTGGTACT  431
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Query  432  GAGAAGTGCTAAACTGTTTCAATGTATTAGGTTTATCCAACTCGTTGAATCTGATTAGTA  491
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Query  492  GTGATCTGTGCTTAGGCTCGGCCTTACAACAACAAACAAAGTTTGACCTCAAATCAGGTA  551
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B

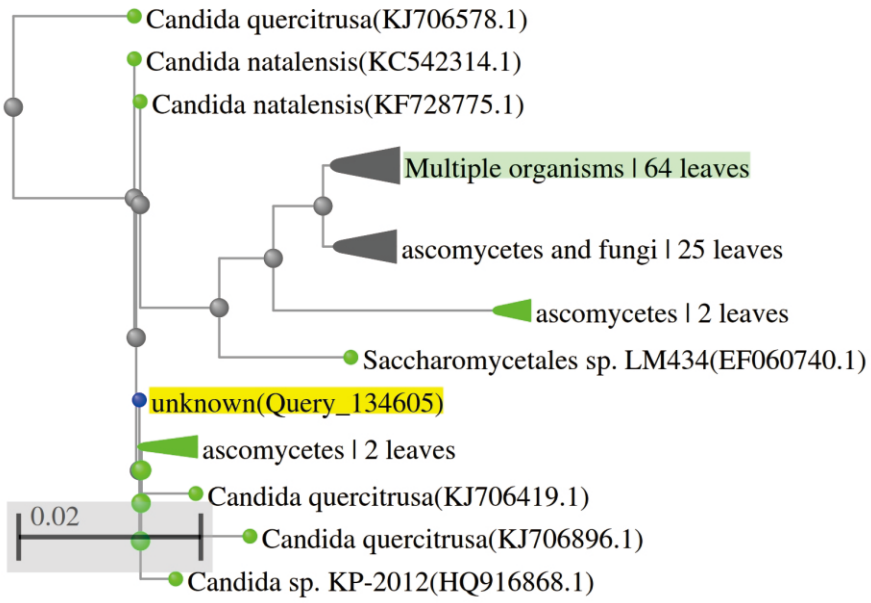

Fig 3 DNA sequence analysis of isolate TK1 with the Primer of ITS 1 (5^{1} - TCCGTAGGTGAACCTGCGG-3^{1}) (A) and phylogenetic tree of isolate TK1 with the Primer of ITS1 (5^{1}-TCCGTAGGTGAACCTGCGG-3^{1}) (B).

A

Range 1: 132 to 695 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
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Query  191  CAATACCAAATCCGAAGATTTGAGAGAGAAATGACGCTCAAACAGGCATGCCCTCTGGAA  250
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Query  311  TTACGTATCGCAATTCGCTGCGTTCTTCATCGATGCGAGAACCAAGAGATCCGTTGTTGA  370
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Query  371  AAGTTTTGAAGATTTTTGAATTTAATCAACAAATTGACTATGGTTAAAATAACAATTCAA  430
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Query  431  TAAAAATATTGAAGTTTAATAAATCTTTGGCCCAACTTGCGCCAGACCAAAGCAATGTTT  490
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B

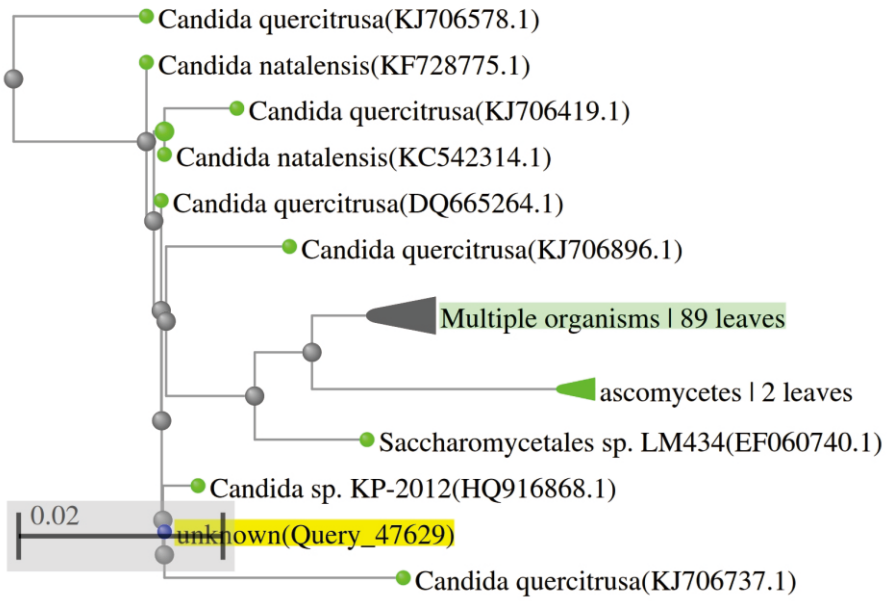

Fig 4 DNA sequence analysis of isolate TK1 with the Primer of ITS 4 (5^{1} - TCCGTAGGTGAACCTGCGG-3^{1}) (A) and phylogenetic tree of isolate TK1 with the Primer of ITS4 (5^{1}-TCCGTAGGTGAACCTGCGG-3^{1}) (B).

Table 1 Results of TK1 Isolate Identification based on Sequence Alignment (BLAST)

Primer	Similarity	Nearest phylogenet ic relative	Strain	Accession number
ITS 1 (5[1] - TCCGTAGGTGAACCTGCGG -3[1])	99%	*Candida natalensis*	B-NC-12-OM13	KF728775.1
ITS 4 (5[1] - TCCTCCGCTTATTGATATGC -3[1])	100%	*Candida quercitrusa*	JHSb	DQ665264.1

20-30% could decrease the yeast growth rate as indicated by the decrease in sediment formed from all isolates. The high concentration of sugar leads to high osmotic pressure which causes low levels of yeast growth (Attfield and Kletsas 2000; Arroyo *et al.* 2013). However, some yeasts possessing the ability to synthesize and utilize glycerol may persist in substrates that have high osmotic pressure due to high sugar concentrations (Myers *et al*1997).

Indigenous Yeast Strain Identification. The isolates of TK1 were selected as the best indigenous yeast isolates with tolerability to alcohol and sugar concentrations in the substrate of 30%. The tolerance capability is indicated by the high absorbance at each level of alcohol and glucose addition, and the consistency of the growth increase from the 0 h to 48 h. The isolate then identified its by rDNA ITS (Internal Transcribed Spacer) region.

The sequence alignment by BLAST showed that TK1 isolates that have the best ability in alcohol and glucose tolerance were similar to *Candida quercitrusa* strain JHSb with 100% similarity. *Candida quercitrusa* has been found as indigenous yeasts from West African cocoa bean and also can be isolated from fruit and vegetables (Jespersen *et al.* 2005; Chanchaichaovivat *et al.* 2007). As shown on the results at Fig. 5, 6 and Table 1., the isolates has nearest known neighbors which is *Candida natalensis* that also near to *Candida anglica* isolated from apple cider with 5% species difference by the following percent nucleotide substitutions in domain D1/D2, however *Candida quercitrusa* have better growth on sucrose (Kurtzman *et al.* 2001).

Candida quercitrusa have several physiological characteristics such the ability to ferment certain sugars semi–anaerobically and assimilate variety carbon compounds as major source carbon in aerobic condition (Kurtzman and Fell 1998). Only sugar such lactose, raffinose and trehalose that cannot ferment by *Candida quercitrusa* with positive D-glucose fermentation, various D-galactose fermentation activities and slow fermentation of sucrose and maltose with the ability to assimilate 19 carbon compounds (Chanchaichaovivat *et al.* 2007).

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REFERENCES

Aatjin A, Lelemboto M, Koapaha T, Mamahit LP. 2013. The utilization of Tacca (*Tacca leontopetaloides*) starch in biscuit product. Jurnal Unsrat. 2(1): 8 p [on line].

Aguilar-Uscanga H, Garcia-Alvarado Y, Gomez-Rodriguez J, Phister T, Delia M, Strehaiano P. 2011. Modelling the growth and ethanol production of *Brettanomyces bruxellensis* at different glucose concentrations. Lett Appl Microbiol. 53: 141-149.

Alexandre H, Charpentier C. 1998. Biochemical aspects of stuck and sluggish fermentation in grape must. J Ind Microbiol Biotechnol. 20: 20-27.

Ali MN, Khan MM. 2014. Screening, identification and characterization of alcohol tolerant potential bioethanol producing yeasts. Curr Res Microbiol Biotechnol. 2(1): 316-324.

Arroyo-Lopez FN, Orlic S, Querol A, Bario E. 2013. Effects of temperature, pH and sugar concentration on the growth parameters of *Saccharomyces cerevisiae*, *S. kudriavzevii* and their interspecific hybrid. Int J Food Microbiol. 131(2-3): 120-127. doi: 10.1016/j.ijfoodmicro.2009.01.035.

Attfield PV, Kletsas S. 2000. Hyperosmotic stress response by strains of bakers' yeasts in high sugar concentration medium. Lett Appl Microbiol. 31(4): 323-327.

Chanchaichaovivat A, Ruenwongsa P, Panijpan B. 2007. Screening and identification of yeast strains from fruits and vegetables: Potential for biological control of post harvest chilli antracnose (*Colleotrichum capsici*). Biol Contr. 42: 326-335. doi: 10.1016/j.biocontrol.2007.

05.016.

Charoenchai C, Fleet GH, Henschke PA. 1998. Effects of temperature, pH and sugar concentration on the growth rates and cell biomass of wine yeasts. Am J Enol Vitic. 49: 283–288.

D'Amato D, Corbo MR, Del Nobile MA, Sinigaglia M. 2006. Effects of temperature, ammonium and glucose concentrations on yeast growth in a model wine system. Int J Food Sci Technol. 41: 1152-1157. doi: 10.1111/j.1365-2621.2005.01128.x

Fakruddin M, Islam MA, Ahmed MM, Chowdhury N. 2013. Process optimization of bioethanol production by stress tolerance yeasts isolated from agro-idustrial wastes. IJSGE. 2(14): 1343-1346.

Ibeas JI, Jimenez J. 1997. Mitochondrial DNA loss caused by ethanol in *S.cereviseae* flour yeasts. Appl Environ Microbiol. 63(1): 7-12.

Jespersen L, Nielsen DS, Honholt S, Jakobsen M. 2005. Occurrence and diversity of yeasts involved in fermentation of West African cocoa beans. FEMS Yeast Research. 5: 441-453.

Kurtzman CP, Fell JW. 1998. The Yeasts: Taxonomic Study, Fourth ed. Elsevier Science, Amsterdam.

Kurtzman, CP, Robnett, CJ, Yarrow D. 2001. Three new species of Candida from apple cider: C. anglica, C.cidri and C.pomicola. Antonie van Leeuwenhoek. 80: 237-244.

Maxwell GRS, Archibong EV, Ogechi N, Chibuzor IA, Nennaya IR, Ogbe SB. 2016. Isolation and identification of local ethanol tolerant yeast populating distillation and milling sites in Nigeria. AJBIO. 4(5): 58-63. doi: 10.11648/j.ajbio.20160405.11.

Moneke AN, Okolo BN, Nweke AI, Ezeogu LI, Ire FS. 2008. Selection and characterization of high ethanol tolerant Saccharomyces yeasts from orchard soil. Afr J Biotechnol. 7(24): 4567-4575. doi: 10.5897/AJB08.933.

Myers D, Lawlor D, Attfield P. 1997. Influence of invertase activity and glycerol synthesis and retention on fermentation of media with a high sugar concentration by *Saccharomyces cerevisiae*. Appl Environ Microbiol. 63(1): 145–150.

Ndouyang CJ, Njitang NY, Facho B, Scher J, Mbofung CMF. 2015. Effect of processing method on the antinutrient content of *Tacca leontopetaloides Kuntze* flour. BJAST. 5(3): 258-269. doi: 10.9734/BJAST/2015/10217.

Ndouyang C, Nguimbo RM, Njitang YN, Scher J, Facho B, Mbofung CMF. 2014. In vivo assessment of the nutritional and subchronic toxicity of *Tacca leontopetaloides* (L.) tubers. Scholarly J Agric Sci. 4(1): 5-13.

Thatoi H, Dash PK, Mohaptra S, Swain MR. 2014. Bioethanol production from tuber crops using fermentation technology: a review. Int J Sust Energy. 35(5): 443-468. doi: 10.1080/14786451.2014.918616.

Tikka C, Osuru HP, Atluri N, Raghavulu PCV, Kumar yellapu N, Mannur IS, Prasad UV, Aluru PS, Varma NK, Bhaskar M. 2013. Isolation and characterization of ethanol tolerant yeast strains. Bioinformation. 9(8): 421-425. doi: 10.6026/97320630009421.

Ukpabi U, Ukenye E, Olojede A. 2009. Raw-material potential of Nigerian wild Polynesian arrowroot (Tacca leontopetaloides) tubers and starch. J Food Technol. 7: 135-138.

Utama GL, Kurnani TBA, Sunardi, Roostita LB. 2016. Isolation and identification of stress tolerance ethanol-fermenting yeasts from mozzarella cheese whey. Int J Adv Sci Eng Info Tech. 6(2): 252-257.

Utama GL, Kurnani TBA, Sunardi, Roostita LB. 2017. Reducing cheese-making by-product disposal through ethanol fermentation and the utilization of distillery waste for fertilizer. Int J GEOMATE. 13(37): 103-107.

You KM, Rosenfield C, Knipple D. 2003. Ethanol tolerance in the yeast *Saccharomyces cerevisiae* is dependent on cellular oleic acid content. Appl Environ Microbiol. 69(3): 1449-1503. doi: 10.1128/AEM.69.3.1499-1503.2003.