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Valorization of crude glycerol into microbial biomass by robust indigenous *saccharomyces cerevisiae* BK isolates

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Abstract

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The rapid expansion of biodiesel production has generated a significant surplus of crude glycerol, necessitating effective valorization strategies. This study aimed to isolate and characterize indigenous yeast strains with exceptional capacity for utilizing crude glycerol as a primary carbon source for microbial biomass production. Eight robust yeast isolates (BK004–BK015), identified as *Saccharomyces cerevisiae* (99.8–100% sequence identity), were obtained from tree canopy soil at Rajabhat Rajanagarindra University, Thailand. Growth performance assays revealed the significant uniqueness of these BK strains; they exhibited remarkable biomass accumulation on crude glycerol (29.60–29.90 g L^{-1}, fresh weight), which was comparable to their growth on glucose. In stark contrast, the reference strain *S. cerevisiae* TISTR 5596 showed markedly limited growth on glycerol, yielding only 3.80 g L^{-1}—a nearly 8-fold difference in efficiency. The BK isolates achieved a biomass productivity (Q_x) of 0.61–0.62 g L^{-1} h^{-1}and an observed fresh biomass yield ($Y_{x/s}$) of 2.97 g g^{-1}, significantly outperforming the reference strain ($Q_x = 0.08$ g L^{-1} h^{-1}; $Y_{x/s} = 0.38$g g^{-1}). While these isolates produced approximately 40 g L^{-1}of bioethanol from glucose, bioethanol production from glycerol was not observed under the tested conditions. This limitation is attributed to the intrinsic NADH/NAD$^+$ redox imbalance in *S. cerevisiae* and the requirement for active respiratory activity to drive glycerol metabolism via the oxidative pathway (GUT pathway). Consequently, glycerol utilization in these indigenous strains is predominantly directed toward biomass synthesis rather than fermentative conversion. Overall, the superior performance of the BK isolates underscores the importance of bioprospecting for 'robust' wild-type strains that possess more efficient metabolic pathways for non-fermentable carbon sources than domesticated laboratory strains, offering a promising platform for sustainable industrial biodiesel byproduct valorization.

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1. Introduction

The escalating global energy demand, coupled with the rapid depletion of conventional fossil fuel reserves, continues to pose a significant challenge to global energy security. Consequently, there has been a profound shift in research focus toward renewable energy systems, among which biodiesel has gained prominence as a highly viable and sustainable alternative. However, the large-scale production of biodiesel inherently generates crude glycerol as its primary byproduct, accounting for approximately 10% to 20% of the total output depending on the reaction efficiency and process type [1 - 3]. The saturation of the market with this byproduct has created an urgent need for effective valorization strategies to ensure the economic sustainability of the biofuel industry [4]. While purified glycerol is a high-value raw material for the cosmetic, pharmaceutical, food, and automotive industries [5]. The high cost of purification technology often makes it economically impractical for small-scale producers to achieve the required purity standards [6, 7].

Consequently, crude glycerol has gained significant traction as a low-cost carbon source for industrial microbiology [8]. However, the inherent variability in its chemical composition—primarily driven by differences in feedstock and transesterification processes—introduces potential inhibitory substances such as residual methanol and high salt concentrations, which can hinder microbial metabolism [9]. Therefore, characterizing the specific crude glycerol profile is essential for evaluating its feasibility as a consistent fermentation substrate. In this work, crude glycerol obtained from a commercial biodiesel plant was utilized, and its major chemical constituents—including glycerol content and potential impurities—were accounted for based on technical specifications to ensure experimental reproducibility [10]. Papanikolaou & Aggelis [11] reported that non-conventional yeasts, such as *Yarrowia lipolytica*, can effectively metabolize industrial glycerol by converting it into glyceraldehyde-3-phosphate for biomass production. While these non-conventional species show promise, *Saccharomyces cerevisiae* remains the preferred host for industrial fermentation due to its robust ethanol tolerance and established processing infrastructure [12].

Hence, this study aims to isolate and characterize *Saccharomyces cerevisiae* strains capable of utilizing crude glycerol as a primary carbon source. The crude glycerol used in this research was sourced from Bangchak Biofuel Co., Ltd., with a concentration of approximately 80%. Traditionally, *S. cerevisiae* fermentation relies on glucose or other high-cost sugars, which can account for up to 50% of total production costs. Although *S. cerevisiae* naturally produces glycerol as an osmoregulant, using external crude glycerol as a sole energy source represents a significant metabolic challenge; however, it also offers a strategic opportunity for cost reduction [13]. Developing a robust process for utilizing crude glycerol as a local, inexpensive feedstock would not only provide a new energy resource but also enhance the circularity of the biodiesel production chain [14]. To address these challenges, this study investigates the targeted isolation of *S. cerevisiae* from soil samples using crude glycerol-enriched media as a selective substrate. This strategy is intended to identify distinct wild strains with inherent metabolic capabilities that overcome the constraints of conventional sugar-based systems. The growth performance and ethanol yield of the selected isolates were systematically evaluated to assess the feasibility of crude glycerol as a cost-effective alternative. The outcomes of this research are anticipated to contribute to the development of specialized yeast platforms that integrate waste valorization with renewable bioenergy production, thereby enhancing the sustainability of the biodiesel–bioethanol value chain.

2. Materials and Methods

Soil Sample Collection

A total of 15 soil samples were collected from beneath the canopies of 15 distinct tree species at Rajabhat Rajanagarindra University, Bang Khla Campus. The sampled species included *Shorea roxburghii*, Mahogany, *Mammea siamensis*, Mango, *Cassia fistula*, Bottlebrush, *Syzygium cumini*, *Jacaranda mimosifolia*, *Dipterocarpus alatus*, *Pterocarpus indicus*, *Delonix regia*, Variegated Indian Rubber, *Acacia auriculiformis*, *Butea monosperma*, and *Samanea saman*. At each site, soil was obtained from the topsoil layer at a depth of approximately 0–10 cm, as this zone typically exhibits the highest microbial activity and organic matter content. To ensure representativeness, soil was excavated from multiple points under the canopy and pooled to form one composite sample per tree. The samples were then stored in sterile plastic bags and transported to the laboratory for further analysis, following the methodology described by Kumla *et al.* [15].

Enrichment and Primary Isolation

One gram of each soil sample was inoculated into 5 mL of an enrichment broth consisting of 2% (w v^{-1}) crude glycerol, 0.3% (w v^{-1}) peptone, and 0.3% (w v^{-1}) yeast extract, with the pH adjusted to 5.6. The crude glycerol used in this study was provided by Bangchak Biofuel Co., Ltd. (Thailand), with its technical specifications and chemical composition summarized in Table 1 [16]. The mixture was contained in 16 × 150 mm test tubes and incubated at 37°C under anaerobic conditions using an anaerobic pouch for 5 days. Modified from Sen *et al.* [17].

Table 1 Technical specifications of the crude glycerol used in this study.

Parameters	Specification
Glycerol content	Min 80% (w w^{-1})
Impurities (Methanol, Salts, Moisture, MONG*)	Max 20% (w w^{-1})
Source	Bangchak Biofuel Co., Ltd., Thailand
Feedstock	Crude Palm Oil (CPO)

Purification and Selection

Following incubation, the cultures were purified using the streak plate method on a solid medium of the same composition (2% crude glycerol, 0.3% peptone, 0.3% yeast extract, and 2% agar, pH 5.6). The plates were incubated anaerobically at 37°C for 5 days. Single yeast colonies with a diameter exceeding 1 mm were selected and re-streaked onto the same medium for a second round of purification under identical conditions.

Subsequently, single colonies larger than 1 mm from the second streak were transferred to the same medium and incubated aerobically at 37°C for 3 days. The isolates were then subcultured onto modified Yeast-Malt (YM) agar, where 1% glucose was replaced with 1% crude glycerol (comprising 1% crude glycerol, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, and 2% (w v^{-1})agar. After aerobic incubation at 37°C for 2 days, the pure colonies were transferred to modified YM agar slants, incubated for an additional 2 days, and finally stored at 4°C for further investigation as shown in Fig. 1.

Identification and Characterization of Yeast Isolates (Conventional identification)

Morphological Characterization

Single colonies grown on modified Yeast-Malt (YM) agar at 37°C for 48 h were examined using a stereo microscope. Colonies exhibiting characteristics typical of *Saccharomyces cerevisiae*—circular shape, smooth margins, approximately 1 mm in diameter, and a cream-white color with a convex center—were selected. Cell morphology was further observed under a light microscope at 10X and 40X magnifications. Isolates showing relatively large ovoid-to-spherical cells and evidence of asexual reproduction via budding were identified as presumptive *S. cerevisiae* for further study.

Fig. 1 Experimental procedure for soil sample collection, enrichment, and selection of *Saccharomyces cerevisiae* isolates.

Sexual Reproduction Analysis

To investigate sexual reproduction, single colonies cultivated on modified YM agar at 37°C for 7–14 days were examined under 10X and 40X magnifications. The presence of sexual spores (ascospores) was evaluated, specifically looking for the formation of 1–4 spherical ascospores contained within an ascus, which is a diagnostic feature of *S. cerevisiae.*

Pseudomycelium Formation Test

The ability of the isolates to form pseudomycelia was tested using the Dalmau method. Single colonies were inoculated onto cornmeal agar plates and incubated at 25°C for 7 days. Following incubation, the presence or absence of pseudomycelial growth was examined microscopically by Kurtzman *et al.* [18].

Aerobic Carbon Source Utilization

The ability of the isolates to utilize various carbon sources under aerobic conditions was evaluated. Yeast cells were inoculated at a density of 1×10^6 cells mL^{-1} into 5 mL of Yeast Nitrogen Base (YNB) broth (0.67% w v^{-1}) supplemented with 0.5% (w v^{-1}) of either glucose, melibiose, or mannitol. The tests were conducted in 16 × 150 mm test tubes and incubated at 37°C under aerobic conditions for 7–21 days. Growth was recorded at 7-day intervals. Isolates capable of utilizing glucose but unable to utilize melibiose and mannitol (typical of *S. cerevisiae*) were selected for subsequent experiments. To further characterize the assimilation profiles, the API kit ID 32 C (Biomerieux, France) was employed. Two-day-old cells suspended in ultra-pure water at a final MacFarland concentration of 2 (250 µL) were inoculated into C-medium, transferred (135 µL) into the wells of the API kit, and incubated at 30°C. Cell turbidity was then monitored at 24, 48, and 72 h post-incubation.

Nitrogen Source Utilization

Nitrogen source utilization was tested following the same procedure as described in carbon source utilization but using Yeast Carbon Base (YCB) broth (1.17% w v^{-1}) as the basal medium. The medium was supplemented with either 0.3% (w v^{-1}) ammonium sulfate or 0.078% (w v^{-1}) potassium nitrate. The cultures were incubated at 37°C under aerobic conditions. Isolates that utilized ammonium sulfate but not potassium nitrate was identified as *S. cerevisiae* candidates.

Anaerobic Fermentation Test

The fermentative capacity of the isolates was determined using seven types of sugars: dextrose (D), maltose (M), sucrose (S), lactose (L), galactose (G), trehalose (T), and raffinose (R). Sugar solutions (2% w v^{-1}) were prepared in distilled water and sterilized at 110°C for 15 minutes. One milliliter of each sugar solution was added to 5 mL of basal yeast fermentation broth (0.45% yeast extract, 0.75% peptone) containing 1% (w v^{-1}) Bromothymol blue. The mixture was contained in 16 × 150 mm tubes equipped with Durham tubes. Each tube was inoculated with 0.2 mL of a 48 h yeast culture and incubated at room temperature for 48 h A positive result was indicated by gas accumulation within the Durham tube and color change of bromothymol blue Wickerham [19].

Biomass Quantification

Eight *S. cerevisiae* strains were cultured in YNB medium supplemented with 1% (v v^{-1}) crude glycerol, 1% (v v^{-1}) pure glycerol, or 1% (w v^{-1}) glucose to determine biomass production. Pre-cultures were grown in 5 mL of YM broth at 37°C for 24 h. Then, a 2% (v v^{-1}) inoculum was transferred to 250-mL flasks containing 50 mL of the respective media. Cultivation was performed at 37°C and 150 rpm for 48 h. Carbon-free medium was used as a negative control. Finally, the biomass was harvested by centrifugation at 8,000 rpm for 5 min, and the productivity was expressed as fresh wet weight (FWW) to evaluate the rapid growth potential of the isolates during the screening phase [20].

Molecular Identification and Phylogenetic Analysis

The selected yeast isolates were subjected to molecular identification through DNA extraction, PCR amplification, and nucleotide sequencing of the D1/D2 domain of the 26S rDNA (large subunit ribosomal DNA), following the protocol described by Kurtzman & Robnett [21] Phylogenetic relationships were reconstructed according to the method of Saitou & Nei [22].

DNA Extraction

Pure yeast cultures were grown on modified YM agar at 37°C for 48 h. A loopful of colonies was suspended in a 1.5 mL microcentrifuge tube containing 200 µL of lysis buffer (100 mM Tris-HCl (pH 8.0), 30 mM EDTA (pH 8.0), and 0.5% SDS). The tubes were secured and boiled for 15 minutes. Subsequently, 200 µL of 2.5 M potassium acetate (pH 7.5) was added, mixed thoroughly, and incubated on ice for 1 h. The mixture was centrifuged at 13,000 rpm for 5 minutes at 4°C. The supernatant was collected and extracted twice with an equal volume of chloroform-isoamyl alcohol. DNA was precipitated by adding an equal volume of cold isopropanol and stored at -20°C for 20 minutes. The DNA pellet was harvested by centrifugation at 14,500 rpm for 16 minutes at 4°C, washed sequentially with 500 µL of 70% ethanol and 500 µL of 99% ethanol. The pellet was dried using a heating block, re-dissolved in 200 µL of deionized water, and stored at -20°C until further use.

PCR Amplification of the D1/D2 Region and Sequencing

The D1/D2 region of the 26S rDNA was amplified using the forward primer NL1 (5'-CATATCAATAAGCGGAGG AAAAG-3') and the reverse primer NL4 (5'-GTCCGTGTTT CAAGACGG-3') Kurtzman *et al.* [23]. The template DNA was diluted to a concentration of 300–800. ng µL^{-1}The 50 µL PCR reaction mixture consisted of 5 µL of 10X Taq buffer, 5 µL of 20 mM $MgCl_2$, 1 µL of 10 mM dNTP mixture, 1 µL each of primers NL1 and NL4 (10 pmol each), 0.3 µL of Taq polymerase (5 U µL^{-1}), 31.7 µL of deionized water, and 5 µL of template DNA. PCR thermal cycling conditions were as follows: initial denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for 20 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 5 minutes. The PCR products were analyzed by 1% agarose gel electrophoresis at 100 V for 35 minutes, stained with ethidium bromide, and visualized under UV light.

The purified PCR products were sequenced and the resulting sequences were compared with those of type strains using pairwise alignment via the EMBOSS Water tool [24] . Phylogenetic trees were constructed using MEGA XI software. Sequences of the isolates and closely related strains retrieved from the BLASTn database were aligned, and a phylogenetic tree was generated using the Neighbor-Joining method. *Lachancea fermentati* NRRL Y-1559^{T} (U84239) was employed as the outgroup. The robustness of the phylogenetic tree was evaluated by bootstrap analysis with 1,000 replicates (Fig. 2).

Evaluation of Bioethanol Production Efficiency

To evaluate bioethanol production, single colonies of the selected *S. cerevisiae* strains were inoculated into 250 mL Erlenmeyer flasks containing 50 mL of YPD medium. The cultures were incubated at 37°C with shaking at 200 rpm for 24 h. Subsequently, a 1% (v v^{-1}) inoculum was transferred into the same medium and cultured under identical conditions for another 24 h to prepare the secondary inoculum.

For the fermentation process, 10% (v v^{-1}) of the yeast culture was inoculated into 50 mL of ethanol fermentation medium containing 10% (w v^{-1}) glycerol as the carbon source (pH 5.6) in 250 mL Erlenmeyer flasks. The fermentation was conducted at 37°C under oxygen-limited conditions by maintaining the flasks in a stationary state (without agitation) and sealing them with rubber stoppers reinforced with parafilm for 48 h. Resultant culture were centrifuged at 4 °C, 15,000 rpm, for 5 min. Supernatants were analyzed for ethanol concentration by gas chromatography (Hewlett-Packard, HP5890 series, USA) with a flame ionization detector at 150 °C using a Porapak QS (cabowax 20 M) column (2 m × 0.32 m) at an oven temperature of 175 °C. Helium at a flow rate of 35 mL/min was used as the carrier gas. as reported in Jutakanoke *et al.* [25] Ethanol yield and ethanol productivity were calculated.

All experiments were performed in triplicate. Data are expressed as mean ± standard deviation. Statistical significance was analyzed using one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$) using SPSS.

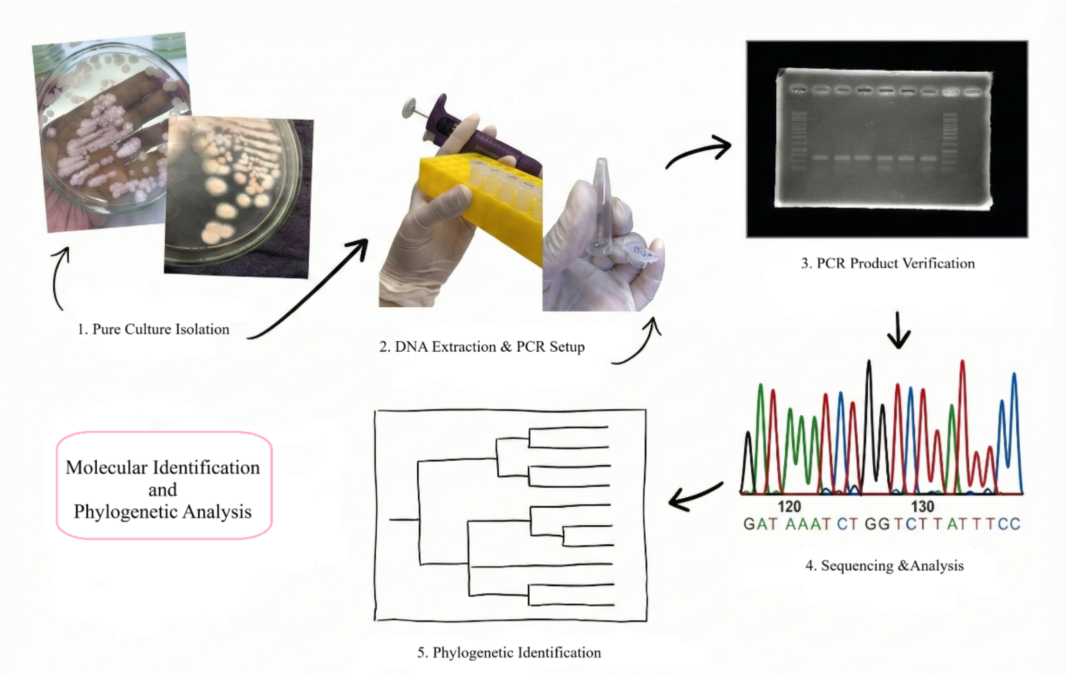

Fig. 2 Systematic workflow for molecular identification and phylogenetic analysis of the isolates.

3. Results and Discussion

Isolation, Screening, and Identification of Yeast from Various Soil Samples

Soil samples were collected from beneath the canopies of 15 different tree species at Rajabhat Rajanagarindra University, Bang Khla Campus. The tree species included *Shorea roxburghii*, Mahogany, *Mammea siamensis*, Mango, *Cassia fistula*, Bottlebrush, *Syzygium cumini*, *Jacaranda mimosifolia*, *Dipterocarpus alatus*, *Pterocarpus indicus*, *Delonix regia*, Variegated Indian Rubber, *Acacia auriculiformis*, *Butea monosperma*, and *Samanea saman*.

Following isolation and screening on culture media using crude glycerol as the primary carbon source, a total of 35 microbial isolates were obtained. After excluding non-yeast microorganisms, the remaining isolates were subjected to primary characterization based on their morphological, reproductive, and physiological properties. Eight isolates were specifically selected as they exhibited characteristics closely resembling those of *Saccharomyces cerevisiae*, as illustrated in Fig. 1 and summarized in Table 2. As shown in Fig. 3, all eight isolated yeast strains exhibited colonies with a diameter of approximately 2 mm on solid culture media. The colonies were characterized by a convex elevation and smooth margins, as illustrated in Fig. 3a. Microscopic observation revealed ovoid cells undergoing asexual reproduction via multilateral budding when cultured in liquid media as demonstrated in Fig. 3b. Furthermore, sexual reproduction was observed through the formation of ascospores under appropriate sporulation-inducing conditions. These ascospores were spherical, ranging from 1 to 4 per ascus, and the asci did not rupture upon reaching maturity as demonstrated in Fig. 3c. These morphological and reproductive features are consistent with the unique characteristics of *Saccharomyces cerevisiae*.

To further confirm their identity, DNA was extracted from the eight isolates, followed by PCR amplification and nucleotide sequencing of the D1/D2 domain of the 26S rDNA gene. The molecular analysis confirmed that all eight isolates were *S. cerevisiae*, with the sequence data summarized in Table 3 the nucleotide sequences of the eight yeast isolates (BK004, BK007, BK008, BK009, BK010, BK011, BK012, and BK015) were compared for sequence similarity against the DNA Data Bank of Japan (DDBJ) database using the BLASTn program. The analysis revealed that all strains exhibited 100% sequence identity to *Saccharomyces cerevisiae*, except for isolate BK010, which showed 99.8% identity. Based on these molecular data, all eight isolates were definitively identified as *S. cerevisiae.*

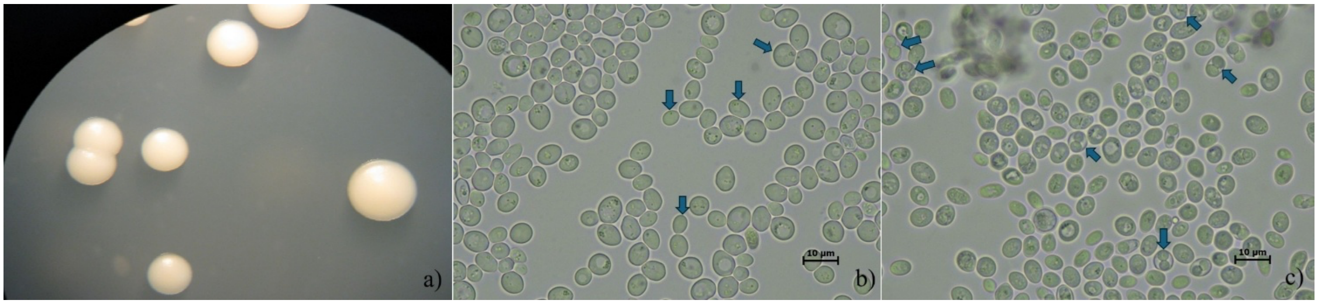

Fig. 3 Morphological features of *S. cerevisiae* on modified YM agar: (a) Macro-morphological appearance of colonies; (b) Micro-morphology (1,000x) showing normal vegetative cells as indicated by the arrows; (c) Presence of ascospores within the asci (arrows), characterized by 1–4 spherical ascospores per ascus.

Molecular Identification and Taxonomic Characterization

The identification process included evaluating the physiological capabilities of the eight *S. cerevisiae* isolates, specifically their ability to utilize various carbon and nitrogen sources under aerobic conditions. These phenotypic characterizations were conducted prior to final molecular taxonomic confirmation. The results of these utilization tests are summarized in Table 3. As shown in Table 4, all eight isolated *S. cerevisiae* strains demonstrated the ability to utilize glucose as a carbon source under aerobic conditions; however, they were unable to assimilate melibiose and mannitol. Regarding nitrogen sources, all strains utilized ammonium sulfate but not potassium nitrate. Their morphological, physiological, and biochemical characteristics are presented in Table 5.

These physiological profiles are entirely consistent with the characteristic properties of reference *S. cerevisiae* type strains as documented in taxonomic. The anaerobic carbon source utilization test for the eight *S. cerevisiae* isolates is presented in Table 6. The results indicated that all isolates were capable of utilizing glucose, sucrose, maltose, and raffinose under anaerobic conditions. In contrast, they were unable to utilize galactose, lactose, and trehalose.

Table 2 Number of *S. cerevisiae* yeast strains isolated from soil under 15 tree species at Rajabhat Rajanagarindra University, Bang Khla.

No.	Tree Species	Family	Sample Code	Number of Strain
1	*Shorea roxburghii* G.Don	Dipterocarpaceae	BK001	-
2	*Swietenia macrophylla* King	Meliaceae	BK002	-
3	*Mammea siamensis* T.Anderson	Guttiferae/Clusiaceae	BK003	-
4	*Mangifera indica* L.	Anacadiaceae	BK004	1
5	*Cassia fistula* L.	Leguminosae/Fabaceae	BK005	-
6	*Callistemon viminalis* (Sol. ex Gaertn.) G.Byrnes	Myrtaceae	BK006	-
7	*Syzygium cumini* (L.) Skeels	Myrtaceae	BK007	1
8	*Jacaranda mimosifolia* D.Don	Bignoniaceaae	BK008	1
9	*Dipterocarpus alatus* Roxb. ex G.Don	Dipterocarpaceae	BK009	1
10	*Pterocarpus indicus* Willd.	Leguminosae/Fabaceae	BK010	1
11	*Delonix regia* (Bojer ex Hook.) Raf.	Leguminosae/Fabaceae	BK011	1
12	*Ficus elastica* Roxb. ex Hornem.	Moraceae	BK012	1
13	*Acacia auriculiformis* A.Cunn. ex Benth.	Leguminosae/Fabaceae	BK013	-
14	*Samanea saman* (Jacq.) Merr.	Leguminosae/Fabaceae	BK014	-
15	*Butea monosperma* (Lam.) Taub.	Leguminosae/Fabaceae	BK015	1

Table 3 Comparison of nucleotide sequences in the NCBI database of representative *S. cerevisiae* yeast strains utilizing crude glycerol as a carbon source, isolated from soil under trees at Rajabhat Rajanagarindra University, Bang Khla.

Strain	Closest phylogenetic relative in the NCBI database	Percentage identity (%)	Query Cover	^{a}Accession number
BK004	*S. cerevisiae* NRRL Y-12632^{T}	100.00	100%	LC834777
BK007	*S. cerevisiae* NRRL Y-12632^{T}	100.00	100%	LC834778
BK008	*S. cerevisiae* NRRL Y-12632^{T}	100.00	100%	LC834779
BK009	*S. cerevisiae* NRRL Y-12632^{T}	99.83	99%	LC834780
BK010	*S. cerevisiae* NRRL Y-12632^{T}	100.00	98%	LC834781
BK011	*S. cerevisiae* NRRL Y-12632^{T}	100.00	99%	LC834782
BK012	*S. cerevisiae* NRRL Y-12632^{T}	100.00	99%	LC834783
BK015	*S. cerevisiae* NRRL Y-12632^{T}	100.00	98%	LC834784

Note: ^{a}Accession number:LSU rRNA (D1/D2) sequences determined in this study and deposited at the DDBJ (DNA Data Bank of Japan) gene databank in Japan

Table 4: Utilization of carbon and nitrogen sources under aerobic conditions of 8 isolated *S. cerevisiae* strains.

Strain	Carbon Source			Nitrogen Source	
	Glucose	Melibiose	Mannitol	Ammonium sulfate	Potassium nitrate
BK004	+	−	−	+	−
BK007	+	−	−	+	−
BK008	+	−	−	+	−
BK009	+	−	−	+	−
BK010	+	−	−	+	−
BK011	+	−	−	+	−
BK012	+	−	−	+	−
BK015	+	−	−	+	−

These fermentative profiles are in full agreement with the standard taxonomic characteristics of *S. cerevisiae* reference strains. The growth performance of the isolated *S. cerevisiae* strains was compared across three different carbon sources, as summarized in Table 7. On glucose medium, all strains exhibited comparable biomass yields ranging from 2.97 to 2.99 g 100 mL^{-1}, with no significant differences observed ($p > 0.05$). Notably, all BK strains demonstrated high efficiency in utilizing both crude and pure glycerol, maintaining biomass levels (2.87–2.99 g 100 mL^{-1}) similar to those obtained on glucose. In contrast, the TISTR strain showed significantly lower growth on both glycerol-based media, yielding only 0.38 g 100 mL^{-1} and 0.37 g 100 mL^{-1} for crude and pure glycerol, respectively ($p > 0.05$). The results of this study demonstrate that the *S. cerevisiae* BK strains (BK004–BK015) possess a remarkable ability to utilize both crude and pure glycerol as carbon sources for biomass production, achieving yields comparable to those obtained from glucose. This observation is particularly significant because most laboratory and industrial strains of *S. cerevisiae* are known to exhibit poor growth on glycerol when compared to glucose, often due to carbon catabolite repression (CCR) or inefficient metabolic flux through the glycerol catabolic pathways (G3P and DHA pathways). The high biomass yield (approximately 2.97–2.99 g 100 mL^{-1}) observed in the BK strains aligns with recent findings highlighting the intraspecies diversity of *S. cerevisiae*. While many wild-type strains struggle to grow on glycerol, recent studies have identified specific isolates with natural variations in the GUT1 (glycerol kinase) and GPD1 genes that enhance glycerol assimilation.

For instance, Strucko *et al.* [26] reported that adaptive laboratory evolution can significantly improve glycerol utilization in *S. cerevisiae*, reaching biomass levels that were previously thought difficult for this species. The superior performance of the BK strains compared to the TISTR reference strain (which produced only ~0.38 g 100 mL^{-1} on glycerol) suggests that the BK series may harbor unique genetic predispositions or superior expression of glycerol transporters, such as Stl1p, which is a proton-symporter crucial for active glycerol uptake under non-fermentative conditions [27]. Furthermore, the ability of BK strains to thrive on crude glycerol without a significant reduction in biomass is a key finding for industrial applications. Crude glycerol, a primary byproduct of biodiesel production, typically contains impurities such as methanol, salts, and soaps that can inhibit microbial growth. The robust growth observed here suggests that BK strains possess high tolerance to these inhibitory compounds. This is consistent with the work of Dobson *et al.* [28], who emphasized the importance of identifying "non-conventional" *S. cerevisiae* strains that can directly convert industrial waste into value-added biomass or products. The comparable yields between pure and crude glycerol in this study indicate that the BK strains are excellent candidates for a sustainable biorefinery model, effectively reducing the costs associated with glycerol purification.

Table 5 Morphological, physiological and biological characteristics of the *S. cerevisiae* isolated from soil under 15 tree species at Rajabhat Rajanagarindra University, Bang Khla.

Characteristic	**BK004**	**BK007**	**BK008**	**BK009**	**BK010**	**BK011**	**BK012**	**BK015**
Cell shape	SO	SO	SO	SO	SO	SO	SO	SO
Budding	M	M	M	M	M	M	M	M
Ascospore	SS	SS	SS	SS	SS	SS	SS	SS
Colony colour	cream	cream	cream	cream	cream	cream	cream	cream
Colony margin	entire	entire	entire	entire	entire	entire	entire	entire
Pseudohyphae	-	-	-	-	-	-	-	-
Growth at 37°C	+	+	+	+	+	+	+	+
Growth at 40°C	-	-	-	-	-	-	+	+
Assimilation of								
N-Acetyl-Glucosamine	-	-	-	-	-	-	-	-
L-Arabinose	-	-	-	-	-	-	-	-
D-Cellobiose	-	-	-	-	-	-	-	-
Cycloheximide(Actidione)	-	-	-	-	-	-	-	-
Vitamin-free	-	-	-	-	-	-	-	-
Ethanol	+	+	+	+	+	+	+	+
D-Galactose	-	+	-	+	-	-	+	+
Glucosamine	-	-	-	-	-	-	-	-
D-Glucose	+	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+
Inositol	-	-	-	-	-	-	-	-
D-Lactose (bovine origin)	-	-	-	-	-	-	-	-
D-Maltose	+	+	+	+	+	+	+	+
D-Mannitol	-	-	-	-	-	-	-	-
D-Melibiose	-	-	-	-	-	-	-	-
Methyl-α-D-Glucopyranoside	-	-	+	-	-	-	-	-
No substrate	-	-	-	-	-	-	-	-
Potassium 2- ketogluconate	-	-	-	-	-	-	-	-
D-Raffinose	+	+	+	+	+	+	+	+
L-Rhamnose	-	-	-	-	-	-	-	-
D-Ribose	-	-	-	-	-	-	-	-
D-Saccharose (sucrose)	+	+	+	+	+	+	+	+
Sodium glucuronate	-	-	-	-	-	-	-	-
D-Sorbitol	-	-	-	-	-	-	-	-
L-Sorbose	-	-	-	-	-	-	-	+
D-Trehalose	+	+	+	+	+	+	+	+
D-Xylose	-	-	-	-	-	-	-	-

Note: SO, Spherical to ovoid; M, Multilateral; SS, Spherical and smooth;

Phylogenetic Analysis Results

The phylogenetic tree presented in Fig. 4 illustrates the evolutionary relationships between the eight yeast isolates and related species. The analysis reveals that all eight isolates (BK004–BK015) cluster within the same clade as the reference strain, *S. cerevisiae* NRRL Y-12632^{NT} (AY048154), exhibiting high sequence similarity. Furthermore, these isolates show close genetic affinity with other recognized reference strains within the genus *Saccharomyces*. Crucially, the isolated strains are clearly phylogenetically distinct from the outgroup, *Lachancea fermentati* NRRL Y-1559^{T} (U84239). Based on these phylogenetic positions and the high bootstrap support, it can be concluded that all eight isolates are definitively identified as *Saccharomyces cerevisiae*.

The successful isolation and identification of eight *S. cerevisiae* strains (BK004–BK015) from soil samples demonstrate that natural environments, particularly rhizosphere soil under diverse tree species, are rich reservoirs for biotechnologically relevant microorganisms. The molecular confirmation using D1/D2 domain sequencing and phylogenetic analysis (Fig. 4) definitively placed these isolates within the *S. cerevisiae* clade, consistent with their morphological and physiological characteristics. A key finding of this study is the superior biomass production of the BK isolates when utilizing glycerol as a carbon source. While *S. cerevisiae* is globally recognized as the primary cell factory for bioethanol production, many industrial and laboratory strains, such as the reference strain TISTR 5596, exhibit poor growth on glycerol

Table 6 Anaerobic utilization of seven carbon sources by the eight isolated *S. cerevisiae* strains. Note: (+) Positive utilization; (–) No utilization.

Strain	**Carbon Source**						
	glucose	galactose	sucrose	maltose	lactose	raffinose	trehalose
BK004	+	−	+	+	−	+	−
BK007	+	−	+	+	−	+	−
BK008	+	−	+	+	−	+	−
BK009	+	−	+	+	−	+	−
BK010	+	−	+	+	−	+	−
BK011	+	−	+	+	−	+	−
BK012	+	−	+	+	−	+	−
BK015	+	−	+	+	−	+	−

Table 7 Biomass concentration of eight *S. cerevisiae* strains using glycerol and glucose as carbon sources.

Strain	**Yeast biomass on different carbon sources (g 100 mL^{-1})**		
	Glucose	**Crude Glycerol**	**Pure Glycerol**
BK004	2.99 (±0.27)^a	2.97 (±0.03)^a	2.89 (±0.17)^a
BK007	2.98 (±0.11)^a	2.96 (±0.02)^a	2.87 (±0.13)^a
BK008	2.99 (±0.30)^a	2.99 (±0.01)^a	2.89 (±0.08)^a
BK009	2.97 (±0.39)^a	2.98 (±0.01)^a	2.89 (±0.14)^a
BK010	2.98 (±0.26)^a	2.99 (±0.01)^a	2.88 (±0.12)^a
BK011	2.99 (±0.21)^a	2.98 (±0.01)^a	2.88 (±0.19)^a
BK012	2.98 (±0.26)^a	2.97 (±0.01)^a	2.87 (±0.20)^a
BK015	2.97 (±0.24)^a	2.99 (±0.02)^a	2.89 (±0.23)^a
TISTR	2.99 (±0.39)^a	0.38 (±0.02)^b	0.37 (±0.06)^b

Noted: Values from triplicate calculations are shown as mean ± standard deviation (SD). Significant differences ($p < 0.05$;DMRT) are indicated by different letters in the same row

Table 8 Biomass production, productivity (Q_x), and observed yield ($Y_{x/s}$) of BK isolates and reference strain after 48 h of cultivation**.**

Yeast Strains	**Substrate (1%)**	**Biomass (gL^{-1})***	**Q_x (gL^{-1}h^{-1})**	**$Y_{x/s}$ (g-fww/g-substrate)**
BK isolates (n=8)	Glucose	29.60-29.90	0.61-0.62	2.98
	Crude Glycerol	29.60-29.90	0.61-0.62	2.97
	Pure Glycerol	28.70-28.90	0.59-0.60	2.88
TISTR	Glucose	29.90	0.62	2.99
	Crude Glycerol	03.80	0.08	0.38
	Pure Glycerol	03.70	0.07	0.37

Noted: **Reported as fresh cell weight*

The biomass accumulation of the eight BK isolates (Table 7) demonstrated no significant difference ($p > 0.05$) when cultivated on glucose or glycerol (crude and pure), with concentrations ranging from 28.7 to 29.9 gL^{-1}. The calculated biomass productivity (Q_x) and observed yield ($Y'_{x/s}$) on crude glycerol were 0.61 to 0.62 g L^{-1} h^{-1}and 2.96 to 2.99 g-fww/g-substrate, respectively (Table 8). These parameters suggest that the BK isolates possess high industrial potential for bioconversion processes. This observation aligns with the well-documented Crabtree-positive nature of *S. cerevisiae*, which typically prioritizes glucose. However, a key finding is the remarkable performance of the BK isolates on glycerol, suggesting a highly active glycerol metabolic pathway, primarily governed by glycerol kinase (GUT1) and mitochondrial glycerol-3-phosphate dehydrogenase (GUT2) [29]. Furthermore, impurities in crude glycerol may act as supplementary nutrients rather than inhibitors. In contrast, the reference strain showed a severe growth reduction (3.7–3.8 g L^{-1}), highlighting the robustness of our local isolates—a trait often found in natural isolates compared to domesticated laboratory strains [30]. While current results focus on biomass, the metabolic flux in these strains is naturally directed toward respiration. To divert this flux toward ethanol production, future strain improvement could focus on the overexpression of the GUT pathway, the deletion of the FPS1 gene to increase internal glycerol concentration, or the strategic use of co-substrates (such as glucose or xylose in small amounts) to maintain the necessary redox balance (NADH/NAD$^+$) during glycerol fermentation, combined with rigorous control of dissolved oxygen levels [30]. We acknowledge that the use of fresh cell weight and initial substrate concentration provides a preliminary assessment; subsequent studies using dry cell weight and residual substrate analysis will further refine these commercial metrics. The isolates obtained from Rajabhat Rajanagarindra University represent promising candidates for industrial applications involving glycerol valorization.

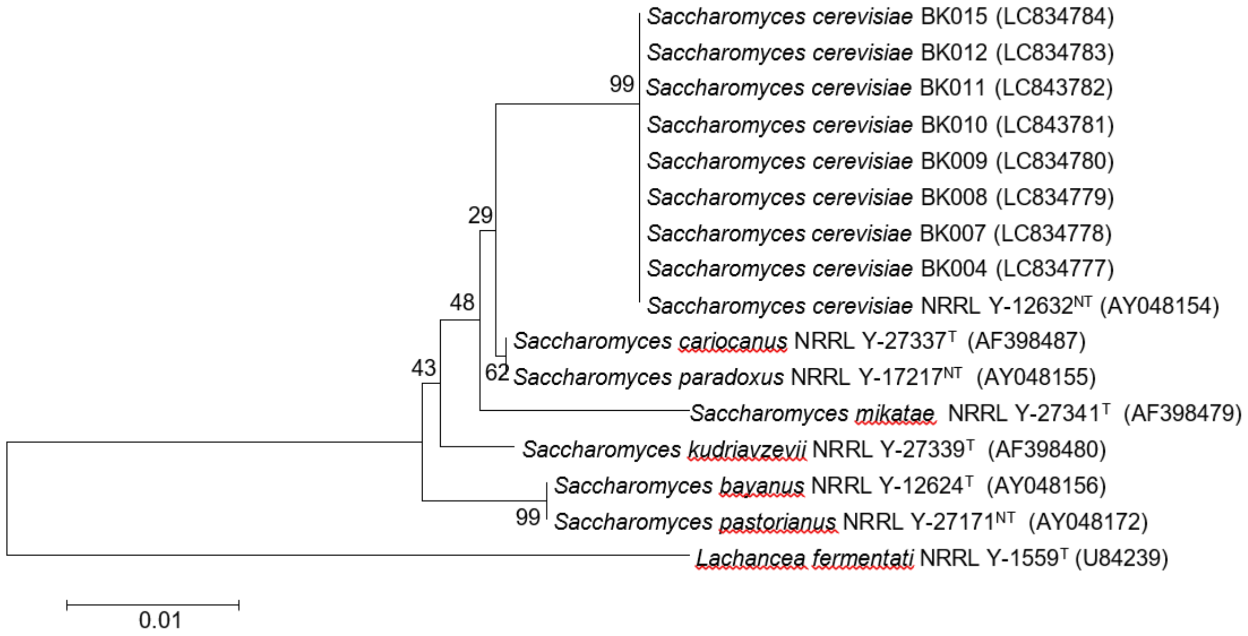

Fig. 4 Phylogenetic trees of *S. cerevisiae* isolate capable of utilizing crude glycerol as a carbon source, isolated from trees at Rajabhat Rajanagarindra University. The tree was constructed using the Neighbor-Joining (NJ) method with 1,000 bootstrap replicates, based on the sequence similarity of the D1/D2 domain of the 26S rDNA, compared with reference strains from the NCBI database.

Evaluation of Bioethanol Production Efficiency

The bioethanol production efficiency of the eight isolated *S. cerevisiae* strains was evaluated using 10% (w v^{-1}) glucose or 10% (w v^{-1}) crude glycerol as carbon sources (Fig. 5). When glucose was used, the BK isolates exhibited ethanol concentrations ranging from 40.2 to 45.6 g L^{-1}. Notably, despite their robust biomass production on glycerol, none of the strains were capable of fermenting glycerol into bioethanol under the specified conditions, yielding negligible ethanol levels (less than 5 g L^{-1}). This result is consistent with the metabolic nature of *S. cerevisiae*, which naturally catabolizes glycerol via the phosphorylative L-G3P pathway. This native pathway consists of a glycerol/H+ symporter (Stl1), a glycerol kinase (Gut1), and an FAD-dependent mitochondrial glycerol-3-phosphate dehydrogenase (Gut2). The Gut2 enzyme is located at the outer face of the inner mitochondrial membrane, where it transfers electrons directly to the mitochondrial respiratory chain. Because this process requires oxygen as the final electron acceptor, the native catabolism of glycerol in wild-type *S. cerevisiae* is considered strictly respiratory [31]. Furthermore, the lack of significant ethanol production can be attributed to the absence of a sufficiently active endogenous DHA pathway. In engineered strains, replacing the native L-G3P pathway with a synthetic NAD+ dependent DHA pathway is typically required to capture electrons in the form of cytosolic NADH, which can then be diverted toward fermentation products. The negligible ethanol yields observed in this study confirm that while the BK isolates possess an efficient respiratory pathway for glycerol assimilation, their fermentative capacity for this substrate is restricted under the current oxygen-limited, non-engineered conditions [32].

In this study, presumptive *Saccharomyces cerevisiae* strains were isolated and screened using traditional taxonomic methods, including morphological characterization, growth profiling on culture media, sexual reproduction analysis, and physiological assays. Following the identification of the genus *Saccharomyces*, species-level confirmation was achieved through nucleotide sequencing of the D1/D2 domain of the 26S rRNA gene and phylogenetic reconstruction. From 15 soil samples collected beneath various tree species at Rajabhat Rajanagarindra University, Bang Khla Campus, a total of 35 microbial isolates were initially obtained using crude glycerol as the primary carbon source. Among these, eight isolates (22.85%) exhibited physiological and biochemical profiles consistent with *S. cerevisiae*. Molecular analysis further confirmed that all eight isolates were indeed *S. cerevisiae*. These findings demonstrate that while natural soils harbor diverse microbial populations, selective pressure using crude glycerol can effectively isolate strains with specific metabolic capabilities.

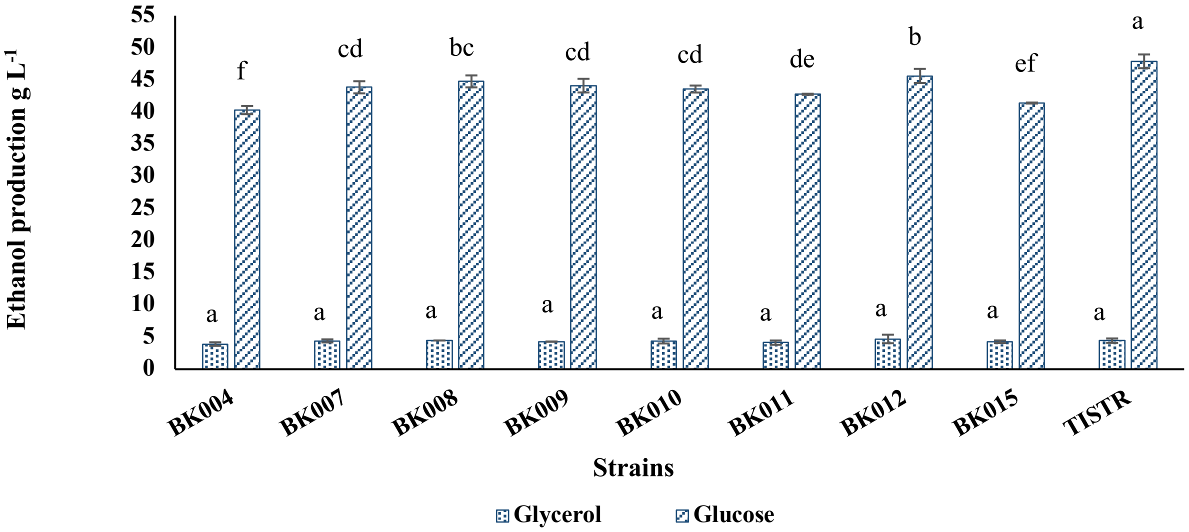

Fig. 5 Bioethanol fermentation results using glycerol and glucose as carbon sources. Data represent the mean of triplicate analyses; error bars indicate the standard deviation (SD). Different letters above bars indicate significant differences ($p < 0.05$).

The distribution of the isolates showed that the majority of *S. cerevisiae* strains were recovered from soil associated with the Leguminosae (Fabaceae) family. However, since 6 out of the 15 sampled trees belonged to this family, a definitive correlation between tree taxa and yeast prevalence remains inconclusive.

The variation in yeast recovery compared to other studies—such as the 124 isolates from mangrove soils reported by Kaewwichian & Khamthaiklang [33] and the 60 isolates from sugarcane fields by Pongcharoen et al. [34]—highlights that yeast density and diversity in soil are highly variable. These differences are driven by multiple factors, including vegetation types, land management, and abiotic parameters such as organic matter content, pH, temperature, moisture, and macronutrients (N, P, K, Na, and Mg) [35]. Furthermore, Botha [36] noted that yeasts are more prevalent in soils under fruit-bearing trees due to the abundance of simple carbohydrates from decaying fruit. The relatively low percentage of *S. cerevisiae* recovery in this study may be attributed to the fact that the sampled trees were non-fruit-bearing and the selection method was highly species-specific. This is consistent with Limtong & Kaewwichian [37], who investigated yeast diversity across various Thai national parks and reported that while Ascomycetous yeasts were common, *S. cerevisiae* members were rarely detected in certain forest soil environments.

The ability of the eight BK isolates to utilize both crude and pure glycerol as a sole carbon source for growth aligns with the findings of Thanito [38], who observed that *S. cerevisiae* could achieve significant cell concentrations on purified glycerol. However, a distinct metabolic divergence was observed in the present study: while the isolates exhibited robust growth (biomass), their bioethanol production was negligible compared to glucose-based fermentation. This limited ethanol production is rooted in a fundamental physiological constraint of *S. cerevisiae*. Glycerol is a more reduced substrate than glucose, and its anaerobic catabolism generates a surplus of NADH that cannot be effectively re-oxidized to NAD+ without an external electron acceptor, creating a metabolic bottleneck. This contrast is stark when compared to specialized glycerol-fermenting systems. For instance, Vikromvarasiri *et al.* [39] utilized a mixed microbial consortium dominated by Gammaproteobacteria (95%), specifically the genera *Enterobacter* and *Klebsiella*. Unlike our yeast strains, these bacteria are naturally equipped for this bioconversion, achieving a maximum ethanol concentration of 11.1 g L^{-1} with a high yield of 0.81–0.85 mol ethanol/mol glycerol. Furthermore, our results differ from oleaginous yeasts like *P. parantarctica* CHC28, which utilize glycerol primarily for lipid accumulation [40]. The metabolic tendency of *S. cerevisiae* BK to favor oxidative pathways for biomass production suggests a strategic pivot for industrial application. Instead of bioethanol, the high efficiency of these strains in converting crude glycerol into Single Cell Protein (SCP) or other high-value compounds presents a viable route for valorizing biodiesel waste. However, moving toward commercial viability requires addressing the impurities in crude glycerol through necessary pretreatment. Ultimately, a comprehensive Techno-Economic Analysis (TEA) is essential to weigh these processing costs against the economic value of the generated biomass, ensuring the transition from glucose to glycerol is both sustainable and profitable [41 - 48].

4. Conclusion

In conclusion, this study successfully isolated and characterized eight robust *Saccharomyces cerevisiae* strains (BK isolates) capable of efficient growth on crude glycerol.

The BK isolates demonstrated superior biomass accumulation, productivity, and yield compared to the reference strain, highlighting their potential for industrial applications where crude glycerol, a biodiesel byproduct, can be valorized into high-value microbial biomass.

Regarding the observed inability of the isolates to ferment glycerol into ethanol, this study suggests that it is likely a combination of intrinsic metabolic characteristics and specific cultivation conditions. Naturally, *S. cerevisiae* is a "non-glycerol fermenting" yeast because it lacks the necessary redox balance to regenerate NAD^+ under anaerobic conditions when using glycerol as a sole carbon source. The high biomass accumulation observed in this study confirms that the metabolic flux was primarily directed toward the oxidative pathway (respiration) under the tested aerobic conditions. Therefore, the lack of ethanol production is not necessarily a defect of the BK isolates but rather a reflection of their native metabolic priority toward biomass synthesis when oxygen is available. To enhance the impact of this research and provide a clear path for future development, several strategies should be explored:

a) Metabolic Engineering: Future efforts will focus on overexpressing the GUT1 and GUT2 genes while introducing or upregulating the dihydroxyacetone (DHA) pathway to enable fermentative glycerol utilization. Deleting the FPS1 aquaglyceroporin could also be investigated to increase intracellular glycerol concentrations.

b) Alternative Valuable Products: beyond bioethanol, the robust growth of these isolates on crude glycerol opens opportunities for producing other high-value metabolites, such as sugar alcohols (e.g., xylitol), organic acids (e.g., succinic acid), or single-cell proteins (SCP) for animal feed.-Process Optimization and Scale-up: Transitioning from shake-flask cultures to controlled bioreactors is essential. Optimizing parameters such as oxygen transfer rates (OTR), pH, and fed-batch feeding strategies will be critical to achieving higher cell densities and steering the metabolism toward specific products.

c) Scale-up Assessment: Future studies will include a techno-economic analysis to evaluate the feasibility of scaling up this process to industrial levels, ensuring that the bioconversion of crude glycerol remains cost-effective and sustainable.

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6. Declaration of Generative AI in Scientific Writing

The use of a generative AI tool in this study was limited to facilitating literature searches and enhancing grammatical accuracy and linguistic clarity.

7. CRediT Author Statement

Nichanun Udomsaksakul: Conceptualization, Methodology, Data curation, Investigation, Writing-Original draft preparation, Visualization, Writing - Reviewing and Editing.

Phakawan Kongchantree: Conceptualization, Validation.

Apiradee Sripiromrak: Methodology, Data curation, Investigation, Visualization.

Napakan Naikhon: Data curation, Supervision.

8. Research Involving Human and Animals Rights

Not applicable

9. Ethics Approval and Consent to Participate

Not applicable

10. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

11. References

[1] Anitha, M., Kamarudin, S. K., & Kofli, N. T. (2016). The potential of glycerol as a value-added commodity. *Chemical Engineering Journal*, *295*, 119–130. https://doi.org/10.1016/j.cej.2016.03.012

[2] Bhuiya, M. M. K., Rasul, M. G., Khan, M. M. K., Ashwath, N., & Azad, A. K. (2016). Prospects of 2nd generation biodiesel as a sustainable fuel—Part: 1 selection of feedstocks, production reviews and case studies. *Renewable and Sustainable Energy Reviews*, *55*, 1109–1128. https://doi.org/10.1016/j.rser.2015.04.163

[3] Karthikeyan, M., Sathish, S., Soosai, M. R., Prabu, D., & Venkatesan, D. (2026). Optimized production of biodiesel from used (waste) cooking oil by utilizing a heterogeneous catalyst, and its studies for sustainable development. *Fuel*, *403*, 136091. https://doi.org/10.1016/j.fuel.2025.136091

[4] Swinnen, S., Klein, M., Carrillo, M., McInnes, J., Nguyen, H. T. T., & Nevoigt, E. (2013). Re-evaluation of glycerol utilization in *Saccharomyces cerevisiae*: Characterization of an isolate that grows on glycerol without supporting supplements. *Biotechnology for Biofuels*, *6*, 157. http://www.biotechnologyforbiofuels.com/content/6/1/157

[5] Tan, T., Özbalci, C., Brügger, B., Rapaport, D., & Dimmer, K. S. (2013). Mcp1 and Mcp2, two novel proteins involved in mitochondrial lipid homeostasis. *Journal of Cell Science*, *126*(16), 3563–3574. https://doi.org/10.1242/jcs.121244

[6] Monteiro, M. R., Kugelmeier, C. L., Pinheiro, R. S., Batalha, M. O., & César, A. d. S. (2018). Glycerol from biodiesel production: Technological paths for sustainability. *Renewable and Sustainable Energy Reviews*, *88*, 109–122.

[7] Yang, F., Hanna, M. A., & Sun, R. (2012). Value added uses for crude glycerol--a byproduct of biodiesel production. *Biotechnology for Biofuels*, *5*(1), 1–10. https://doi.org/10.1186/1754-6834-5-13

[8] Russmayer, H., Egermeier, M., Kalemasi, D., & Sauer, M. (2019). Spotlight on biodiversity of microbial cell factories for glycerol conversion. *Biotechnology Advances, 37*, 107395. https://doi.org/10.1016/j.biotechadv.2019.05.001

[9] Hansen, C. F., Hernandez, A., Mullan, B. P., Moore, K., Trezona-Murray, M., King, R. H., & Pluske, J. R. (2009). A chemical analysis of samples of crude glycerol from the production of biodiesel in Australia, and the effects of feeding crude glycerol to growing-finishing pigs on performance, plasma metabolites and meat quality at slaughter. *Australian Journal of Experimental Agriculture*, *49*(2), 154–161. https://doi.org/10.1071/EA08210

[10] Kaur, J., Sarma, A. K., Jha, M. K., & Gera, P. (2020). Valorisation of crude glycerol to value-added products: Perspectives of process technology, economics and environmental issues. *Biotechnology Reports*, *27*, e00487. https://doi.org/10.1016/j.btre.2020.e00487

[11] Papanikolaou, S., &Aggelis, G. (2002). Lipid production by *Yarrowia lipolytica* growing on industrial glycerol in a single-stage continuous culture. *Bioresource Technology, 82*(1), 43-49.

[12] Yahyazadeh, A., Bot, A., Nanda, S., & Dalai, A. K. (2023). Technological insights on glycerol valorization into propanediol through thermocatalytic and synthetic biology approaches. *Fermentation*, *9*(10), 894. https://doi.org/10.3390/fermentation9100894

[13] Perpelea, A., Wijaya, A. W., Martins, L. C., Rippert, D., Klein, M., Angelov, A., Peltonen, K., Teleki, A., Liebl, W., Richard, P., Thevelein, J. M., Takors, R., Sá-Correia, I., & Nevoigt, E. (2022). Towards valorization of pectin-rich agro-industrial residues: Engineering of *Saccharomyces cerevisiae* for co-fermentation of D-galacturonic acid and glycerol. *Metabolic Engineering*, *69*, 1–14. https://doi.org/10.1016/j.ymben.2021.10.001

[14] Guragain, Y. N., & Vadlani, P. V. (2021). Renewable biomass utilization: A way forward to establish sustainable chemical and processing industries. *Clean Technologies*, *3*(1), 243–259. https://doi.org/10.3390/cleantechnol3010014

[15] Kumla, J., Nundaeng, S., Suwannarach, N., & Lumyong, S. (2020). Evaluation of multifarious plant growth promoting trials of yeast isolated from the soil of Assam tea (*Camellia sinensis* var. *assamica*) plantations in northern Thailand. *Microorganisms*, *8*(8), 1168. https://doi.org/10.3390/microorganisms8081168

[16] UAC Global Public Company Limited. (2014, February 24). *Annual report 2014: Energy Transformation for Future* [Annual report]. http://www.uac.co.th/

[17] Sen, B., Dabir, A. P., Lanjekar, V. B., & Ranade, D. R. (2015). Isolation and partial characterization of a new strain of *Klebsiella pneumoniae* capable of high 1,3 propanediol production from glycerol. *Global Journal of Environmental Science and Management, 1*(2), 99–108. https://doi.org/10.7508/gjesm.2015.02.001

[18] Kurtzman, C. P., Fell, J. M., & Boekhout, T. (2011). *The yeasts: A taxonomic study* (5th ed.). Elsevier.

[19] Wickerham, L. J. (1951). *Taxonomy of the yeasts* (Technical Bulletin No. 1029). U. S. Department of Agriculture.

[20] Stanbury, P. F., Whitaker, A., & Hall, S. J. (2017). *Principles of fermentation technology* (3rd ed.). Butterworth-Heinemann.

[21] Kurtzman, C. P., & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeast from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek*, *73*(4), 331–371. https://doi.org/10.1023/A:1001761008817

[22] Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

[23] Kurtzman, C. P., Boekhout, T., Robert, V., Fell, J. W., & Deak, T. (2003). Methods to identify yeasts. In T. Boekhout & V. Robert (Eds.), *Yeasts in food: Beneficial and detrimental aspects* (pp. 69–121). B.Behr's Verlag.

[24] Rice, P., Longden, I., & Bleasby, A. (2000). EMBOSS: The European Molecular Biology Open Software Suite. *Trends in Genetics*, *16*(6), 276–277. https://doi.org/10.1016/S0168-9525(00)02024-2

[25] Jutakanoke, R., Leepipatpiboon, N., Tolieng, V., Kitpreechavanich, V., Srinorakutara, T., & Akaracharanya, A. (2012). Sugarcane leaves: Pretreatment and ethanol fermentation by *Saccharomyces cerevisiae*. *Biomass and Bioenergy*, *39*, 283–289. https://doi.org/10.1016/j.biombioe.2012.01.018

[26] Strucko, T., Zirngibl, K., Tharwat Tolba Mohamed, E., Feist, A., Patil, K. R., & Förster, J. (2015). Development of an efficient glycerol-utilizing *Saccharomyces cerevisiae* strain via adaptive laboratory evolution. In Jacobsen, C. S. (Ed.), *Danish Microbiological Society Annual Congress 2015* (p. 22). Danish Microbiological Society.

[27] Dmytruk, K., Semkiv, M., & Sibirny, A. (2025). Glycerol bioconversion to biofuel and value-added products by yeasts. *FEMS Yeast Research*, *25*, foaf038. https://doi.org/10.1093/femsyr/foaf038

[28] Dobson, R., Gray, V., & Rumbold, K. (2012). Microbial utilization of crude glycerol for the production of value-added products. *Journal of Industrial Microbiology & Biotechnology*, *39*, 217–226. https://doi.org/10.1007/s10295-011-1038-0

[29] Yu, Z., Chang, Z., Lu, Y., & Xiao, H. (2023). Metabolic engineering of *Saccharomyces cerevisiae* for glycerol utilization. *FEMS Yeast Research*, *23*, 1–8. https://doi.org/10.1093/femsyr/foad014

[30] Pirzadi, Z., & Meshkani, F. (2022). From glycerol production to its value-added uses: A critical review. *Fuel*, *329*, 125044. https://doi.org/10.1016/j.fuel.2022.125044

[31] Xiberras, J., Klein, M., & Nevoigt, E. (2019). Glycerol as a substrate for *Saccharomyces cerevisiae* based bioprocesses – Knowledge gaps regarding the central carbon catabolism of this 'non-fermentable' carbon source. *Biotechnology Advances*, *37*, 107378. https://doi.org/10.1016/j.biotechadv.2019.03.017

[32] Klein, M., Swinnen, S., Thevelein, J. M., & Nevoigt, E. (2017). Glycerol metabolism and its exploitation in *Saccharomyces cerevisiae*. *Metabolic Engineering*, *38*, 364–372. https://doi.org/10.1016/j.meteno.2016.09.001

[33] Kaewwichian, R., & Khamthiklang, S. (2017). Yeast in mangrove forest soil from the central Thailand and it ability in degradation of starch, carboxymethylcellulose and xylan. *Burapha Science Journal, 22*, 411-422.

[34] Pongcharoen, P., Chawneua, J., & Tawong, W. (2018). High temperature alcoholic fermentation by new thermotolerant yeast strains *Pichia kudriavzevii* isolated from sugarcane field soil. *Agriculture and Natural Resources, 52*, 511-518.

[35] Yurkov, A. M. (2018). Yeasts of the soil-obscure but precious. *Yeast, 35*(5), 369-378.

[36] Botha, A. (2011). The Importance and ecology of yeast in soil. *Soil Biology and Biochemistry*, *43*, 1-8.

[37] Limtong, S., & Kaewwichian, R. (2009). *Diversity of yeast in soil from national parks in the north eastern part of Thailand and their role in degradation of organic matters in soil* (Final report). Department of Microbiology, Faculty of Science, Kasetsart University.

[38] Thanito, K. (2016, July 23). Cultivation of *S. cerevisiae* using glycerol from biodiesel process in laboratory.

Proceedings of the 4th National and 2nd International Academic Conference 2016, Bangkok Thonburi University, 164-171.

[39] Vikromvarasiri, N., Laothanachareon, T., Champreda, V., & Pisutpaisal, N. (2014). Bioethanol production from glycerol by mixed culture system. *Energy Procedia*, *61*, 1213–1218. https://doi.org/10.1016/j.egypro.2014.11.1059

[40] Rakkitkanphun, C., Lomthong, T., Wanlapa, A., Teeka, J., & Areesirisuk, A. (2019). Effect of chemical and physical factors on oleaginous yeast cultivation by using biodiesel-derived crude glycerol as a main carbon source. *Nakhon Sawan Rajabhat University Science and Technology Journal*, *11*(13), 65-78.

[41] Atabani, A. E., Silitonga, A. S., Badruddin, I. A., Mahlia, T. M. I., Masjuki, H. H., & Mekhilef, S. (2012). A comprehensive review on biodiesel as an alternative energy resource and its characteristics. *Renewable and Sustainable Energy Reviews*, *16*(4), 2070–2093. https://doi.org/10.1016/j.rser.2012.01.003

[42] Bansod, Y., Ghasemzadeh, K., & D'Agostino, C. (2025). Techno-economic assessment of biodiesel-derived crude glycerol purification processes. *RSC Sustainability*, *3*(6), 2605–2618. https://doi.org/10.1039/d4su00599f

[43] Selvakumari, I. A., Jayamuthunagai, J., & Bharathiraja, B. (2025). Techno-economic analysis and feasibility study of malic acid production using crude glycerol from biodiesel refineries. *Polish Journal of Environmental Studies*. https://doi.org/10.15244/pjoes/211640

[44] Attarbachi, T., Kingsley, M., & Spallina, V. (2024). Experimental scale-up and technoeconomic assessment of low-grade glycerol purification from waste-based biorefinery. *Industrial & Engineering Chemistry Research, 63*(11),4905–4917. https://doi.org/10.1021/acs.iecr.3c03868

[45] Yu, J., Dong, J., Zhang, C., & Xiao, D. (2014). Effect of *GPD1* and *GPD2* deletion on the production of glycerol and ethanol in the yeast *Saccharomyces cerevisiae*. In T.C. Zhang, P. Ouyang, S. Kaplan, & B. Skarnes (Eds.), *The 2012 International Conference on Applied Biotechnology (ICAB 2012)* (Vol. 2, pp. 171–179). Springer. https://doi.org/10.1007/978-3-642-37922-2_17

[46] Swinnen, S., Ho, P. W., Klein, M., & Nevoigt, E. (2016). Genetic determinants for enhanced glycerol growth of *Saccharomyces cerevisiae*. *Metabolic Engineering*, *36*, 68–79. https://doi.org/10.1016/j.ymben.2016.03.003

[47] Khattab, S. M. R., Katahira, M., & Watanabe, T. (2025). Engineering *Saccharomyces cerevisiae* for ethanol production from glycerol, xylose, acetic acid, and glucose. *Bioresource Technology*, *435*, 132921.

[48] Nakanishi, A., Zhang, K., Matsumoto, R., & Yamamoto, N. (2022). Estimation of carbon metabolism in *Saccharomyces cerevisiae* acclimatized to glycerol assimilation with quantitative PCR. *Microorganisms*, *10*(6),1173. https://doi.org/10.3390/microorganisms10061173