

Enhanced Lipid Production from *Zygosaccharomyces siamensis* AP1 by UV Mutagenesis and Cerulenin Selection

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Abstract. *Yeast-derived single-cell oils (SCOs) are promising renewable lipid sources for biodiesel and high-value bioproducts. Among oleaginous yeasts, Zygosaccharomyces siamensis AP1, isolated from Indonesian wild bee honey, demonstrates significant potential for lipid production; however, further improvement is needed for industrial-scale applications. This study aimed to enhance lipid accumulation in Z. siamensis AP1 via ultraviolet (UV) mutagenesis, followed by selective screening with cerulenin and ethanol/hydrogen peroxide (H₂O₂) stress. The yeast underwent repeated UV exposure to achieve 60% lethality. The surviving cells were then screened on media with increasing concentrations of cerulenin (5–160 μmol/L) or ethanol/H₂O₂ (2–4% v/v, 1–2 mmol/L). Lipid-producing mutants were characterized for growth kinetics, lipid content, and fatty acid profiles. Among the 43 mutants obtained, the cerulenin-resistant strain CR15 exhibited superior performance, reaching 28.6% lipid content after 96 hours, an increase from 24.6% at 72 hours. At the same, the wild type and the oxidative-stress mutant (MT19) showed reduced lipid accumulation over time. The wild-type lipid content decreased from 22.86% at 72 hours to 18.53% at 96 hours, while lipid content from strain MT19 decreased from 24.64% at 72 hours to 20.09% at 96 hours. Gas chromatography analysis revealed that CR15 produced a diverse fatty acid profile with both even- (arachidonic acid (16%), eicosadienoic acid (5.88%), gondoic acid (18.70%), linoleic acid (26.63%), palmitoleic acid (11.89%) and odd-chain (margaric acid (14.46%)) fatty acids, suggesting mutations in fatty acid synthase (FAS) enzyme function. In contrast, MT19 primarily produced linoleic acid (45.75%) as an adaptive response to oxidative stress. These results indicate that UV mutagenesis coupled with cerulenin selection can effectively enhance lipid biosynthesis and alter fatty acid composition in Z. siamensis. The CR15 mutant represents a promising candidate for advancing sustainable microbial oil production. Further transcriptomic analysis is suggested to clarify the genetic basis of these metabolic changes.*

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INTRODUCTION

Yeast-derived lipids are promising sustainable alternatives for biodiesel, food additives, cosmetics, and pharmaceuticals (Bandhu et al., 2020; Beopoulos & Nicaud, 2012; Lee et al., 2024; Szczepańska et al., 2022). Their controlled fermentation enables consistent quality independent of agricultural land and seasonal variations (Beopoulos & Nicaud, 2012). Yeast biorefineries utilizing agro-industrial wastes can produce bio-oils alongside value-added co-products, enhancing economic viability (Cosío-Cuadros et al., 2023; Llamas et al., 2020; Samavi & Rakshit, 2022). Oleaginous yeasts accumulate over 20% of their dry cell weight as lipids (Qian et al., 2023; Wusnah et al., 2023). Key species include *Yarrowia lipolytica* (Beopoulos et al., 2010; Szczepańska et al., 2022), *Rhodotorula species* (Babau, 2015; Martinez-Silveira et al., 2019), *Lipomyces starkeyi* (Saha et al., 2025), and *Rhodosporidium* sp. and *Trichosporon* sp. (Bandhu et al., 2020; Sajish et al., 2022).

Indonesia's biodiversity offers novel microorganisms with biotechnological potential (Kanti, 2022; Ikhwan et al., 2023). Wild bee honey harbors osmotolerant yeasts adapted to high sugar concentrations (Kanti, 2022). The isolation of oleaginous *Zygosaccharomyces siamensis* AP1 from wild bee honey in Ampana, Central Sulawesi (Anjani & Ilmi, 2018), exemplifies this potential. *Zygosaccharomyces* sp. exhibit remarkable stress tolerance, including osmotolerance and moderate ethanol tolerance (Csoma et al., 2023; Jagtap et al., 2022), making them suitable for industrial bioprocesses. *Zygosaccharomyces lentus* tolerates up to 8% (v/v) ethanol and high osmotic pressure (Csoma et al., 2023), traits that are advantageous for lipid production.

UV mutagenesis remains a cost-effective approach for strain improvement (Choi et al., 2015; Saravanan et al., 2017; Soedarmodjo et al., 2019). It induces random mutations, creating genetic diversity without requiring detailed genetic knowledge (Choi et al., 2015). UV mutagenesis improves lipid production through: (1) redirecting carbon flux by downregulating competing pathways; (2) enhancing NADPH supply; (3) suppressing competing carbon sinks; (4) increasing lipogenic enzyme activity; and (5) inducing stress-responsive signaling cascades (Ji et al., 2024; Lim et al., 2015; Liu et al., 2015; Muthuraj et al., 2019; Sivaramakrishnan & Incharoensakdi, 2023; Teco-Bravo et al., 2019; Tensingh & Shankar, 2022; Vardar-Yel et al., 2024; Wen et al., 2025; Yamada et al., 2017). Examples include *Schizochytrium* sp. mutants with 40.3% increased DHA production (Wen et al., 2025), *Saccharomyces cerevisiae* mutants achieving 44% cellular lipid content (Ji et al., 2024), and *Chlorella* sp. mutants with lipid content increases from 34% to 48% (Muthuraj et al., 2019; Liu et al., 2015).

Effective screening methods are critical for identifying desired mutants (Bao et al., 2021; Cao et al., 2022). Selective screening imposes constraints that couple metabolic activity to cell fitness (Akhgari et al., 2022; Chen et al., 2024; Raman et al., 2014). Cerulenin inhibits fatty acid synthesis by targeting fatty acid synthase (Bao et al., 2021; Kataya et al., 2025), while oxidative stress agents like ethanol and hydrogen peroxide affect membrane lipid composition (Bonatto, 2020; Santos et al., 2012; Sekova et al., 2020). Combining mutagenesis with multi-step selective screening increases the likelihood of isolating high-lipid-producing mutants (Chen et al., 2024; Raman et al., 2014).

Despite the industrial potential of *Zygosaccharomyces* sp. and the documented success of UV mutagenesis in improving lipid production, the application of UV mutagenesis to *Z. siamensis* remains unexplored. Previous work demonstrated that *Z. siamensis* AP1 possesses

inherent lipid-producing capability (Ilmi & Siswontoro, 2021; Ilmi et al., 2023; Salsabila & Ilmi, 2021), but productivity may be insufficient for industrial applications. This study aimed to enhance the lipid production capacity of *Z. siamensis* AP1 through UV mutagenesis combined with selective screening using cerulenin and ethanol/hydrogen peroxide mixture.

MATERIALS AND METHODS

Yeast Strain

The wild-type yeast strain *Zygosaccharomyces siamensis* AP1 was isolated from wild bee honey collected in Ampana, Sulawesi, Indonesia (Anjani & Ilmi, 2018). The mutant strains (MTxx and CRxx; Table 1) were generated by mutagenesis in this study. All strains are maintained in the Laboratory of Microbiology at the Faculty of Biology, Universitas Gadjah Mada.

Random Mutation of Strain AP1 Using Ultraviolet Radiation

Mutagenesis of strain AP1 was performed according to Yamada et al. (Yamada et al., 2017). Briefly, the yeast was grown in an Erlenmeyer flask containing 50 mL Yeast Peptone Dextrose (YPD [HiMedia]) for 24h at 28°C and 200 rpm agitation. The cells were then transferred into a sterile Petri dish and irradiated with an ultraviolet (UV) lamp 15 W [Sankyo Denki G15T8] using a wavelength of 253.7 nm until achieving 60% cell death. Surviving cells were incubated until the cells' density (OD_{600}) reached 1. The cells were transferred into YPD containing increasing concentrations of cerulenin [Sigma-Aldrich] (5 – 160 $\mu\text{mol/L}$) as a selective agent, then incubated until $OD_{600} = 2$. UV mutagenesis was then repeated as described above. UV mutagenesis was also conducted using a mixture of ethanol [Merck] and hydrogen peroxide (H_2O_2) [Merck] as a selective agent at two concentrations: 2% (v/v) with 1 mmol/L and 4% (v/v) with 2 mmol/L. The surviving mutants from the last cycles were kept in YPD with agar [Oxoid] (15 g/L) containing the highest corresponding selective agent concentration. Mutant strains from cerulenin selection were given CR as strain code, while mutant strains from ethanol/ H_2O_2 selection were coded MT (Table 1).

Lipid Production Characterization

All mutant strains were assayed for lipid content by growing them in 50 mL of Nitrogen-Limited Medium (NLM) containing glucose [Millipore] (100 g/L), peptone [Millipore] (3 g/L), and yeast extract [Oxoid] (8 g/L). The flasks were incubated for 48h in a rotary shaker [B-One] at 28°C and 200 rpm agitation. Strains with the highest lipid content from each UV mutagenesis with different selective agents were further characterized by measuring cell density every 24h for 96h at $\lambda=600$ nm, along with their biomass and lipid content at 72 and 96h.

Biomass was measured gravimetrically by harvesting cells via centrifugation, washing with distilled water, drying at 70°C to constant weight, and weighing to determine the dry cell weight per mL of fermentation medium. Lipid amount was determined using the Bligh and Dyer method (Bligh & Dyer, 1959), in which cells were homogenized with chloroform: methanol (1:2, v/v), followed by the addition of chloroform and water to achieve a final ratio of 2:2:1.8 (v/v/v) for phase separation. The lower chloroform phase containing extracted lipids was recovered, evaporated, and weighed to determine total lipid mass per mL of fermentation medium. Lipid content was expressed as a percentage of lipid amount per biomass dry cell weight (Eq. 1).

$$\text{Lipid content (\%)} = \frac{\text{Lipid amount (g/mL)}}{\text{Dry cell biomass (g/mL)}} \times 100 \quad \text{Eq.1}$$

Lipid Profile Analysis

Lipids produced from the selected mutants after 96h in NLM were analyzed using a gas chromatography method. Samples were methylated before injection, as described by Ichihara and Fukubayashi (2010). Afterward, 1 μL of methylated sample was injected into GC [Agilent 7890B] using HP-88 column (100 m length) and He as carrier gas. Column temperature was set initially at 100 $^{\circ}\text{C}$ and held for 5 minutes, then increased to 240 $^{\circ}\text{C}$ (4 $^{\circ}\text{C}$ per minute) and held for 15 minutes. Detector temperature was set to 260 $^{\circ}\text{C}$. External standards were used as references.

Data Analysis

Data were analyzed and visualized using Microsoft Excel 2019 [Microsoft]. GC chromatograms were analyzed using ChemStation Software [Agilent]. Data comparisons between groups were performed statistically using paired-samples t-tests ($\alpha = 0.05$) in JASP (ver. 0.95.3).

RESULTS AND DISCUSSION

Random Mutation of *Zygosaccharomyces siamensis* AP1 Using Ultraviolet Irradiation

Mutagenesis of *Z. siamensis* AP1 was performed using a 15 W UV lamp with a wavelength of 253.7 nm. The surviving mutants were then screened using either cerulenin or a mixture of ethanol and hydrogen peroxide (H_2O_2). A total of 43 mutants were obtained, comprising 18 from the cerulenin screening and 25 from the ethanol and H_2O_2 screening (Table 1). Four mutants produced high lipid content (CR15, 52.8%; CR25, 20%; MT17, 13.1%; and MT19, 16.6%), while two other mutants (MT29 and MT34) did not accumulate any detectable lipid (Figure 1). The best mutants selected from the cerulenin screen had higher lipid content than the 19% reported for the wild-type strain in a previous study (Anjani & Ilmi, 2018). In contrast, the best mutants selected from the ethanol and H_2O_2 screen produced lower lipid content than the wild-type.

Table 1. Mutant strains obtained from UV-mutagenesis of *Z. siamensis*

Strains from cerulenin screening	Strains from ethanol/ H_2O_2 screening
CR11, CR12, CR13, CR14, CR15, CR16, CR17, CR18, CR19, CR20, CR21, CR22, CR23, CR24, CR25, CR26, CR27, CR28	MT11, MT12, MT13, MT14, MT15, MT16, MT17, MT18, MT19, MT20, MT21, MT22, MT23, MT24, MT25, MT26, MT27, MT28, MT29, MT30, MT31, MT32, MT33, MT34, MT35

Lipid synthesis in fungi involves several key enzymes, including ATP: citrate lyase, malate dehydrogenase, acetyl-CoA carboxylase, and fatty acid synthase (Ratledge, 2014). Due to the complexity of these pathways, random mutagenesis is a suitable approach for strain engineering, given its simplicity and rapidity. However, an efficient screening method is required to isolate the desired mutants. In this study, we used cerulenin and a mixture of ethanol and H_2O_2 as screening agents.

The cerulenin screening in our study identified mutants with higher lipid content, consistent

with previous studies on *Lipomyces starkeyii* (Tapia et al., 2012) and *Rhodospiridium toruloides* (Yamada et al., 2017). Cerulenin is a known antibiotic that inhibits lipid synthesis in fungi (Ono et al., 1974) and bacteria (Heath et al., 2001). It specifically inhibits the β -ketoacyl synthase component of FAS, restricting de novo synthesis of saturated fatty acids and directing cellular metabolism toward desaturation and storage pathways; hence, mutants surviving cerulenin selection can produce high amounts of lipid (Ratledge, 2014; Tanaka et al., 1976; Wang et al., 2009).

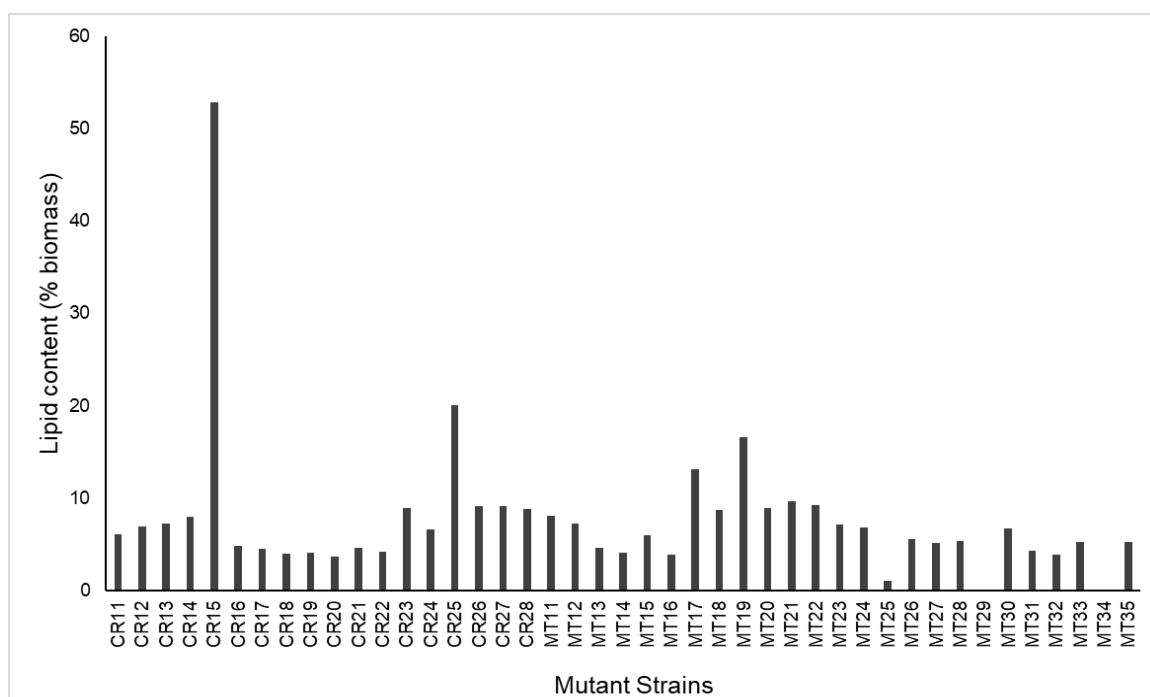


Figure 1. Mutant strains and their lipid contents after grown in Nitrogen Limited Medium (NLM) for 24h. with incubation temperature 28°C and 200 rpm agitation. CRs are mutant strains from mutation using cerulenin as a selective agent, MTs are mutant strains from mutation using ethanol/H₂O₂ as a selective agent.

Ethanol and H₂O₂, on the other hand, are environmental stressors that reduce membrane integrity and induce ROS-mediated oxidative stress, altering membrane lipid composition (Ma et al., 2013; Yang et al., 2012). Similar to cerulenin, mutants with enhanced lipid production can withstand the effects of ethanol and H₂O₂ (Wu et al., 2020); however, the increase is moderate compared to cerulenin-screened mutants, as shown by our result (Figure 1) and confirmed by Guo et al. (2019) and Yamada et al. (2017). Hence, cerulenin proved to be a more effective selection agent than the ethanol/H₂O₂ mixture.

Growth Profiles of Chosen Mutants

Growth profiles were performed on the mutants with the highest lipid content from each screening method, namely CR15 from the cerulenin screening and MT19 from the ethanol/H₂O₂ screening. Mutants and wild-type (AP1) strains were grown in NLM medium for 96 h. Cell density was measured every 24 hours using a spectrophotometer at an A600. The plot of cell absorbance values against time is shown in Figure 2.

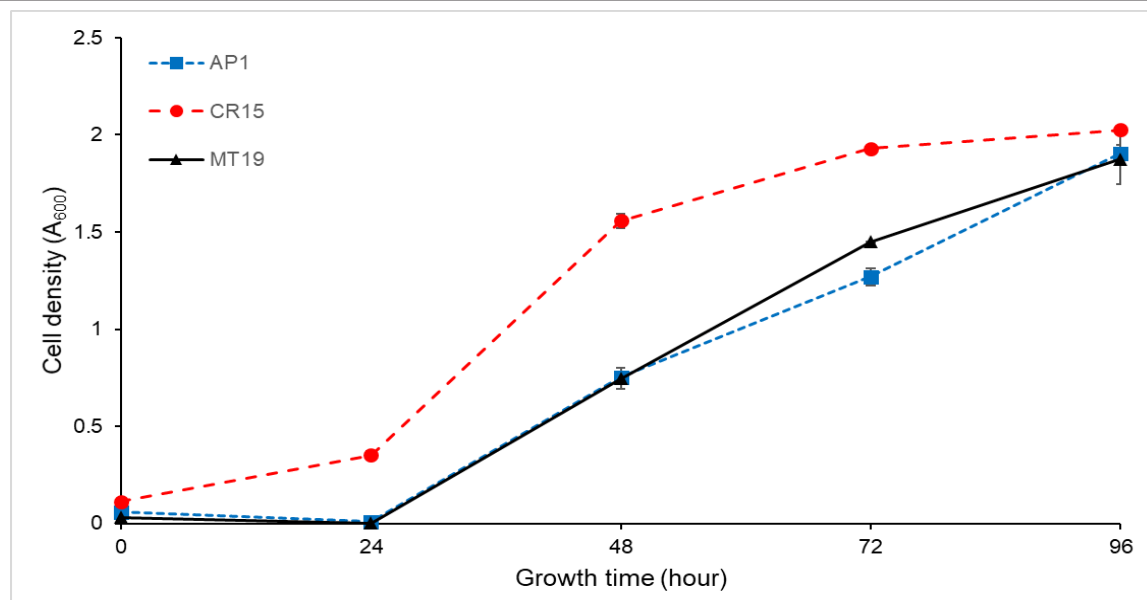


Figure 2. Growth curve of wildtype strain *Zygosaccharomyces siamensis* AP1 and the selected mutants (CR15 and MT19) in Nitrogen Limited Medium (NLM) for 96h. The incubation temperature was 28 °C with 200 rpm agitation

The CR15 strain exhibited an accelerated growth profile, achieving a cell density (OD_{600}) of 0.35 at 24 hours, compared to 0.05 for both the AP1 and MT19 strains. This early growth advantage was maintained throughout the cultivation period, with CR15 reaching a maximum cell density (OD_{600}) of 2.05 at 96 hours, representing an 8% increase over the wildtype strain. Higher growth performance of CR15 following cerulenin selection suggests that UV mutagenesis altered its lipid biosynthesis pathways. Cerulenin specifically inhibits acetyl-CoA carboxylase (ACC1) and fatty acid synthase (FAS1/FAS2), which are rate-limiting enzymes in fatty acid synthesis (Omura, 1976; Wakil et al., 1983). Surviving mutants from cerulenin may possess an enhanced fatty acid synthesis capacity through the overexpression or modified kinetics of ACC1 and FAS enzymes, alternative lipid synthesis pathways that bypass cerulenin-sensitive steps, or improved lipid salvage mechanisms to maintain membrane integrity with reduced de novo synthesis (Henry et al., 2012; Tehlivets et al., 2007). These lipid-synthesis adaptations likely conferred a metabolic advantage to CR15 under the nitrogen-limited fermentation conditions used in this study. Nitrogen limitation is a well-established trigger of lipid accumulation in oleaginous yeasts: when nitrogen is depleted in the presence of excess carbon, the reduced demand for amino acid and protein synthesis reroutes carbon flux into triacylglycerol (TAG) storage (Calvey et al., 2016; Kerkhoven et al., 2016). Mechanistically, nitrogen exhaustion elevates AMP deaminase activity, lowering cellular AMP levels and reducing NADH-dependent isocitrate dehydrogenase (ICDH) activity in mitochondria, leading to citrate accumulation and export to the cytosol (Gluth et al., 2025; Kerkhoven et al., 2016). Cytosolic citrate is then cleaved by ATP: citrate lyase (ACL) to generate acetyl-CoA, the primary precursor for fatty acid biosynthesis, with NADPH supplied via the pentose phosphate pathway (Zhang et al., 2016). Consequently, nitrogen limitation simultaneously suppresses biomass and protein synthesis while actively driving carbon into lipid storage through the adenylate–citrate–ACL axis (Calvey et al., 2016; Zhang et al., 2016). CR15, with its cerulenin-

selected lipid synthesis adaptations, was likely better positioned to exploit this nitrogen-limited carbon overflow, thereby achieving higher lipid accumulation and, consequently, greater growth performance than the wild-type under these conditions. On the other hand, the similar growth performance of MT19 and the wild-type AP1 suggests that MT19's oxidative stress adaptations do not provide significant advantages under nitrogen limitation, confirming the specificity of the cerulenin selection effect.

Lipid Production Characteristics of Chosen Mutants

Lipid production characteristics of the wild-type strain and chosen mutant strains were determined after 72 and 96 hours of growth in NLM medium. At each time point, cell and lipid biomass were measured gravimetrically, and the resulting weights were compared within strains. The results are presented in Figure 3. The results show that after 72 hours, all three strains produced similar biomass (Figure 3A), although AP1 produced slightly higher biomass (1.1 g/L) than CR15 and MT19 (0.99 and 0.97 g/L, respectively). However, at the 96th hour, the biomass of CR15 is significantly lower ($\alpha = 0.05$) than that of AP1 and MT19, and it has only increased slightly from its biomass at the 72nd hour. Lipid productions from all strains, on the other hand, are statistically similar either after 72 or 96 hours of production (Figure 3B). Lipid contents calculated based on total biomass and lipid amount (Figure 3C) show that CR15 lipid content increased from 24.6% at 72nd hour to 28.6% at 96th hour. In contrast, the lipid content of AP1 and MT19 decreased after 96 hours of production, from 22.9 to 18.5% and from 24.6 to 20%, respectively.

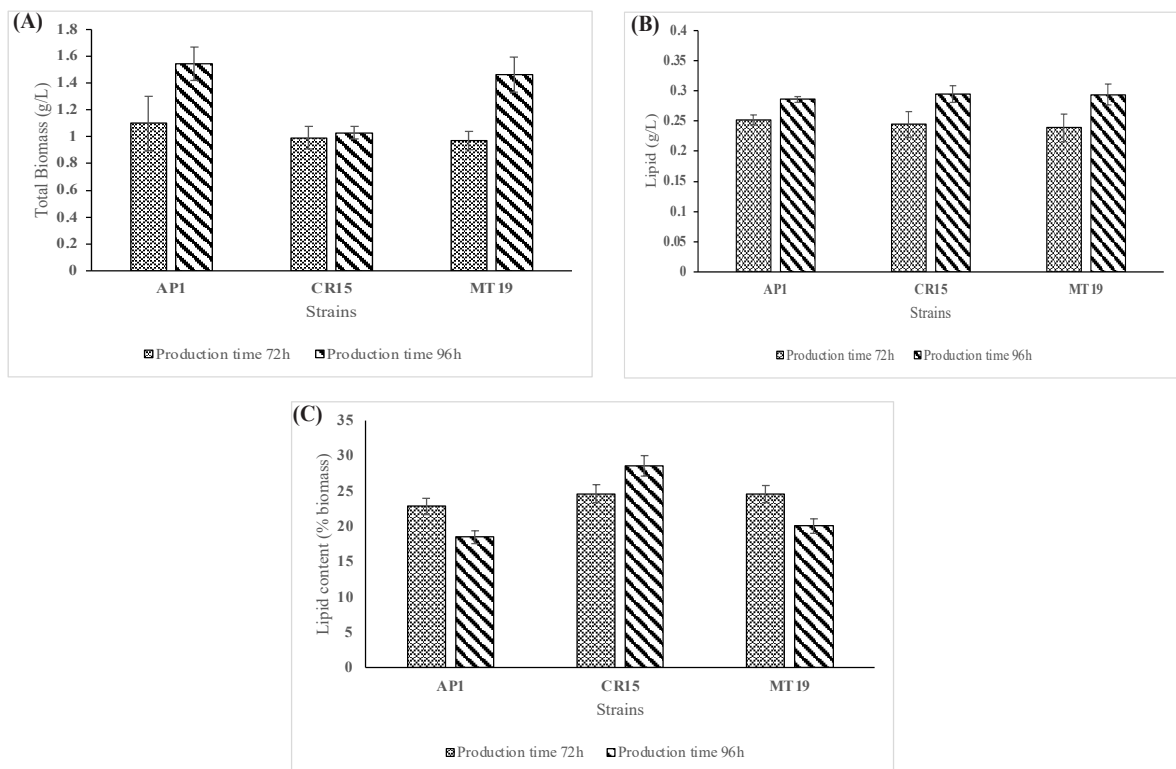


Figure 3. Biomass (A), lipid (B), and lipid content (C) of the wild-type *Zygosaccharomyces siamensis* AP1 strain and selected mutants (CR15 and MT19) grown for 72 and 96 hours in a Nitrogen-Limited Medium (NLM) at 28°C with 200 rpm agitation

The increased lipid content in CR15 suggests that the strain blocks lipid remobilization. The blockage can be caused by limited N in the growth medium, as also shown by previous studies in *Candida* sp. 107 (Gill et al., 1977) and *Yarrowia lipolytica* (Lazar et al., 2018). Proteomic analysis by Shi et al. (2013) revealed that oleaginous yeast downregulates β -oxidation and lipase proteins during nitrogen-limited accumulation phases. Another factor suspected of causing remobilization blockage is genetic modification resulting from UV mutagenesis followed by cerulenin selection. Cerulenin resistance involves not only FAS modification but also broader metabolic adaptations affecting lipid homeostasis (Yamada et al., 2017). High lipid production, which was shown by CR15 during the screening (Figure 1) and production (Figure 3) phases, can only occur when β -oxidation is disrupted. Otherwise, newly synthesized lipids were rapidly catabolized, as observed in the AP1 and MT19 strains (Leber et al., 2016). Total biomass from both strains increased along with lipid amounts (Figure 3), indicating that accumulated lipid was utilized as a carbon source for cell growth. However, further transcriptomic analyses are needed to prove those suspicions.

Based on the results, CR15 was selected as the optimal strain due to superior lipid accumulation during the 96-hour cultivation period. While all three candidate strains demonstrated oleaginous characteristics, CR15 exhibited a progressive increase in lipid content from 24.6% at 72 hours to 28.6% at 96 hours, representing a 16.3% improvement in lipid accumulation capacity during extended cultivation. In contrast, AP1 and MT19 showed declining lipid content trajectories, decreasing to 18.5% and 20.0%, respectively, by 96 hours. Lipid content and lipid productivity are established as the primary selection criteria for identifying superior oleaginous strains for biofuel production, as demonstrated by previous studies in microalgae (Griffiths and Harrison, 2009; Nascimento et al., 2013; Rodolfi et al., 2009) and oleaginous microorganisms (Vello et al., 2014). Griffiths and Harrison (2009) demonstrated that lipid productivity is the key characteristic for selecting algal species for biodiesel production, emphasizing that strains with higher lipid accumulation rates offer superior economic viability. Similarly, Nascimento et al. (2013) validated lipid productivity and lipid content as the principal selection criteria for screening microalgae strains, noting that these parameters directly determine the commercial feasibility of developing biodiesel feedstock. Rodolfi et al. (2009) further confirmed that lipid-content-guided strain selection, combined with productivity assessments, enables the identification of optimal biocatalysts for large-scale oil production. The superior and sustained lipid accumulation profile of CR15, particularly its capacity to continue increasing lipid content beyond 72 hours while competitor strains declined, positions it as the most promising candidate for scaled biodiesel production processes (Vello et al., 2014).

Fatty Acid Profiles of Chosen Mutants

Fatty acid profiles of lipids produced by the wild-type strain (AP1) and mutant strains (CR15 and MT19) were analyzed using Gas Chromatography (Agilent 7890B) with an HP-88 column (100 m length) and He as the carrier gas. The resulting chromatograms were analyzed using ChemStation Software. The fatty acid composition of the lipid samples is shown in Figure 4. The results show that the fatty acid composition of the lipids from CR15 and MT19 shifted from that of AP1. Notable fatty acid changes from CR15 are linoleic acid (26.63%), arachidonic acid (16%), margaric acid (14.46%), gondoic acid (12.71%), and palmitoleic acid (11.89%). On the other hand, MT19 fatty acid composition is less diverse. Three major fatty acids produced by the strain are linoleic acid (45.75%), margaric acid (22.78%), and gondoic acid (10%). Other fatty

acids are very low, less than 10%.

A mixture of even-chain (C16, C18, C20) and odd-chain (C17) fatty acids from CR15 lipid indicates cerulenin selection favors strains with mutated FAS enzymes, which accept alternative substrates (propionyl-CoA alongside acetyl-CoA) (Arai et al., 1982; Qiao et al., 2023) and altered chain-length determination (Heil et al., 2019; Qin et al., 2023). Cerulenin is known to block FAS enzyme activity and reduce lipid synthesis in cells (Henry et al., 2012; Tehlivets et al., 2007). Hence, modifications to fatty acid synthesis pathways create multiple independent mechanisms that enable the cell to produce enough lipids to overcome the cerulenin block.

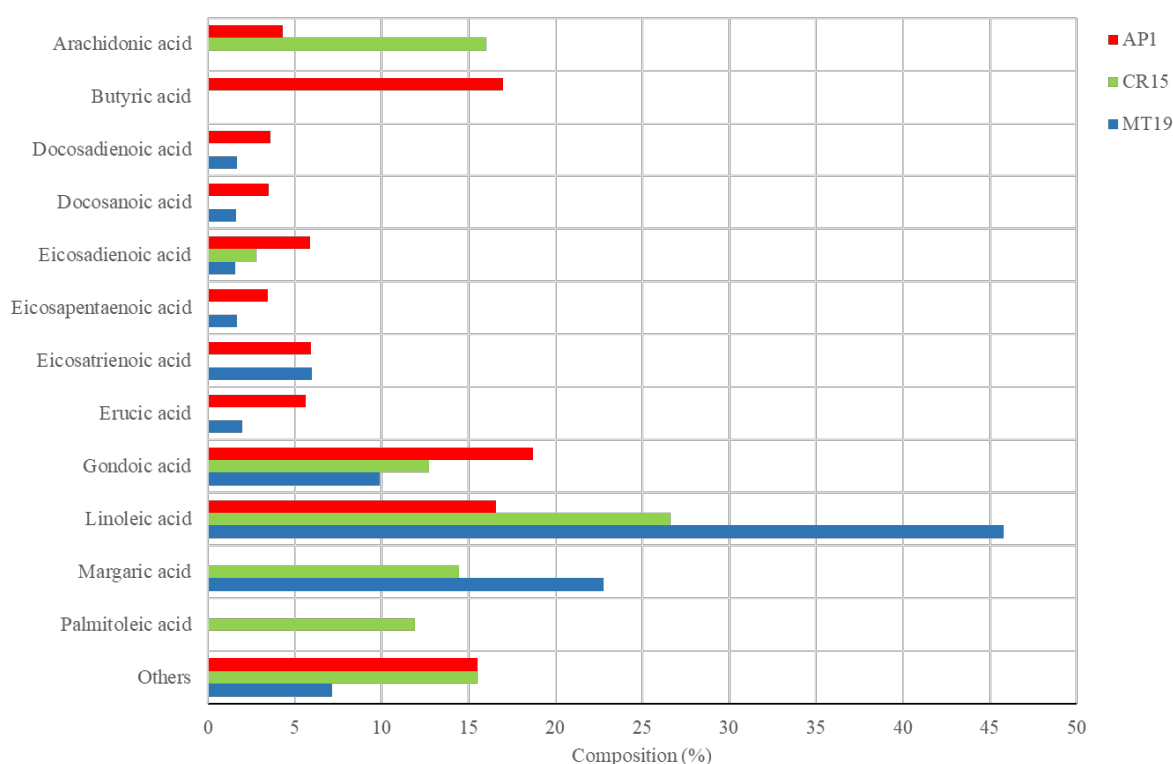


Figure 4. Fatty acid compositions of the wild-type *Zygosaccharomyces siamensis* API strain and selected mutants (CR15 and MT19) after being grown for 96 hours in a Nitrogen-Limited Medium (NLM) at 28 °C with 200 rpm agitation. Analyses were done using Gas Chromatography (GC) with HP-88 column

A different fatty acid profile is observed from MT19; the lipid contains mainly unsaturated C18:2 linoleic acid. This sharp increase suggests oxidative stress created by ethanol and peroxide causes membrane lipid peroxidation and protein damage, creating a specific biophysical problem that requires a specific lipid solution. We suspected that oxidative stress favored mutants with increased expression of acetyl-CoA carboxylase (ACC1) and acyl-CoA desaturase (OLE1), which, in turn, increase linoleic acid synthesis. This causes remodeling of cell membrane components as described by Wang et al. (2015). Although a previous study reported that PUFA-producing yeasts are more sensitive to oxidative stress (Cipak et al., 2008), a recent study showed that high PUFA concentrations can increase membrane fluidity and enhance yeast adaptation to oxidative stress (Vázquez et al., 2019).

CONCLUSION

Based on the study, UV mutagenesis was successfully used to modify *Z. siamensis* AP1, with cerulenin proving more effective than an ethanol/H₂O₂ mixture as a selective agent. The best cerulenin-screened mutant, CR15, not only showed an accelerated growth profile but also demonstrated superior lipid accumulation, increasing lipid content between 72 and 96 hours (from 24.6% to 28.6%), unlike the wild-type and MT19 strains. This suggests the mutation blocked lipid remobilization. Furthermore, the selection method altered the fatty acid composition of CR15, producing a diverse mix of even- and odd-chain fatty acids, indicating mutated FAS enzymes. On the other hand, the ethanol/H₂O₂ mutant (MT19) adapted to oxidative stress by producing a high concentration of linoleic acid (45.75%). Further study is suggested to determine the effect of UV mutagenesis on the transcription of genes in the lipid synthesis pathway.

AUTHOR CONTRIBUTION

M.I. designed the study, analyzed the data, and wrote the manuscript. **C.E.B.S.** did the experiments and analyzed the data.

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CONFLICT OF INTEREST

The authors state there is no conflict of interest regarding the research or its funding.

REFERENCES

- Akhgari, A., Baral, B., Koroleva, A., Makitrynsky, R., Brameyer, S., Koshla, O., Zotchev, S. B., Jung, K., Mironov, V., & Bechthold, A. (2022). Single cell mutant selection for metabolic engineering of actinomycetes. *Metabolic Engineering*, 73, 148-158. DOI: <https://doi.org/10.1016/j.ymben.2022.07.002>
- Anjani, K. D., & Ilmi, M. (2018). Screening of oleaginous yeast isolates from flower nectar and wild honey. *Jurnal Mikologi Indonesia*, 2(2), 99–111. DOI: <https://doi.org/10.46638/jmi.v2i2.42>
- Arai, K., Kawaguchi, A., Saito, Y., Koike, N., Seyama, Y., Yamakawa, T., & Okuda, S. (1982). Propionyl-Coa induced synthesis of even-chain-length fatty acids by fatty acid synthetase from *Brevibacterium ammoniagenes*. *Journal of Biochemistry*, 91(1), 11–18. DOI: <https://doi.org/10.1093/oxfordjournals.jbchem.a133667>
- Babau, P. M. (2015). Growth and lipid accumulation of the yeast *Rhodotorula glutinis* (*Rhodospiridium toruloides*) from glucose, xylose and glycerol : towards agricultural and industrial byproduct utilization for lipid production for energy use [*Doctoral dissertation*]. University of Toulouse.

- Bandhu, S., Srivastava, A., Ghosh, D., & Chaudhuri, T. K. (2020). Yeast Single Cell Oils from Bioresources: Current Developments in Production and Applications. *Current Sustainable/ Renewable Energy Reports*, 7(4), 109–120. DOI: <https://doi.org/10.1007/s40518-020-00160-6>
- Bao, X., Koorengel, M. C., Groot Koerkamp, M. J. A., Homavar, A., Weijn, A., Crielaard, S., Renne, M. F., Lorent, J. H., Geerts, W. J., Surma, M. A., Mari, M., Holstege, F. C. P., Klose, C., & de Kroon, A. I. P. M. (2021). Shortening of membrane lipid acyl chains compensates for phosphatidylcholine deficiency in choline-auxotroph yeast. *The EMBO Journal*, 40(20), e107966. DOI: <https://doi.org/10.15252/embj.2021107966>
- Beopoulos, A., Desfougères, T., Sabirova, J., & Nicaud, J. M. (2010). *Yarrowia lipolytica* as a Cell Factory for Oleochemical Biotechnology. In K. N. Timmis (Ed.), *Handbook of hydrocarbon and lipid microbiology* (pp. 3003–3010). Springer Berlin Heidelberg. DOI: https://doi.org/10.1007/978-3-540-77587-4_223
- Beopoulos, A., & Nicaud, J.-M. (2012). Yeast: A new oil producer? *OCL*, 19(1), 22–28. DOI: <https://doi.org/10.1051/ocl.2012.0426>
- Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911–917. DOI: <https://doi.org/10.1139/o59-099>
- Bonatto, D. (2020). Rethinking the role of lipids in lager yeast cells during beer fermentation from a transcriptome and systems biology perspective. *bioRxiv*, 2020-01. DOI: <https://doi.org/10.1101/2020.01.28.922898>
- Calvey, C. H., Su, Y. K., Willis, L. B., McGee, M., & Jeffries, T. W. (2016). Nitrogen limitation, oxygen limitation, and lipid accumulation in *Lipomyces starkeyi*. *Bioresource technology*, 200, 780-788. DOI: <https://doi.org/10.1016/j.biortech.2015.10.104>
- Cao, M., Tran, V. G., Qin, J., Olson, A., Mishra, S., Schultz, J. C., Huang, C., Xie, D., & Zhao, H. (2022). Metabolic engineering of oleaginous yeast *Rhodotorula toruloides* for overproduction of triacetic acid lactone. *Biotechnology and Bioengineering*, 119(9), 2529–2540. DOI: <https://doi.org/10.1002/bit.28159>
- Chen, S., Yang, Z., Zhong, Z., Chen, H., Chen, M., Xu, S., Huang, J., Wu, Y., Li, Y., Wang, J., & Rao, Z. (2024). Ultrahigh-throughput screening-assisted in vivo directed evolution for enzyme engineering. *Biotechnology for Biofuels and Bioproducts*, 17(1), 9. DOI: <https://doi.org/10.1186/s13068-024-02457-w>
- Choi, S.-J., Park, H.-J., & Lee, J.-H. (2015). Isolation of *Chlorella vulgaris* Mutants Producing High Lipid and their Characterization. *Applied Chemistry for Engineering*, 26(5), 533–537. DOI: <https://doi.org/10.14478/ace.2014.1135>
- Cipak, A., Jaganjac, M., Tehlivets, O., Kohlwein, S. D., & Zarkovic, N. (2008). Adaptation to oxidative stress induced by polyunsaturated fatty acids in yeast. *Biochimica et Biophysica Acta*, 1781(6–7), 283–287. DOI: <https://doi.org/10.1016/j.bbalip.2008.03.010>
- Cosío-Cuadros, R., Núñez-López, G., Martha F. Martín del Campo, Rodríguez, J. A., Mateos-Díaz, J. C., & Sandoval, G. (2023). *Agro-Industrial Wastes to Sustainable Bio-Oil Fuels, Enzymes and Biobased Chemicals in Yeast-Biorefineries*. CRC Press.
- Csoma, H., Acs-Szabo, L., Papp, L. A., Kállai, Z., Miklós, I., & Sipiczki, M. (2023). Characterization of *Zygosaccharomyces lentus* Yeast in Hungarian Botrytized Wines. *Microorganisms*, 11(4), 852. DOI: <https://doi.org/10.3390/microorganisms11040852>

- Gill, C. O., Hall, M. J., & Ratledge, C. (1977). Lipid accumulation in an oleaginous yeast (*Candida* 107) growing on glucose in single-stage continuous culture. *Applied and Environmental Microbiology*, 33(2), 231–239. DOI: <https://doi.org/10.1128/aem.33.2.231-239.1977>
- Gluth, A., Czajka, J. J., Li, X., Bloodsworth, K. J., Eder, J. G., Kyle, J. E., ... & Zhang, T. (2025). Nitrogen limitation causes a seismic shift in redox state and phosphorylation of proteins implicated in carbon flux and lipidome remodeling in *Rhodotorula toruloides*. *Biotechnology for Biofuels and Bioproducts*, 18(1), 80. <https://doi.org/10.1186/s13068-025-02657-y>
- Griffiths, M. J., & Harrison, S. T. L. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology*, 21(5), 493–507. DOI: <https://doi.org/10.1007/S10811-008-9392-7>
- Guo, M., Cheng, S., Chen, G., & Chen, J. (2019). Improvement of lipid production in oleaginous yeast *Rhodospiridium toruloides* by ultraviolet mutagenesis. *Engineering in Life Sciences*, 19(8), 548–556. DOI: <https://doi.org/10.1002/elsc.201800203>
- Heath, R. J., White, S. W., & Rock, C. O. (2001). Lipid biosynthesis as a target for antibacterial agents. *Progress in Lipid Research*, 40(6), 467–497. DOI: [https://doi.org/10.1016/S0163-7827\(01\)00012-1](https://doi.org/10.1016/S0163-7827(01)00012-1)
- Heil, C. S., Wehrheim, S. S., Paithankar, K. S., & Grininger, M. (2019). Fatty Acid Biosynthesis: Chain-Length Regulation and Control. *Chembiochem*, 20(18), 2298–2321. DOI: <https://doi.org/10.1002/cbic.201800809>
- Henry, S. A., Kohlwein, S. D., & Carman, G. M. (2012). Metabolism and regulation of glycerolipids in the yeast *Saccharomyces cerevisiae*. *Genetics*, 190(2), 317–349. DOI: <https://doi.org/10.1534/genetics.111.130286>
- Ichihara, K., & Fukubayashi, Y. (2010). Preparation of fatty acid methyl esters for gas-liquid chromatography. *Journal of Lipid Research*, 51(3), 635–640. DOI: <https://doi.org/10.1194/jlr.D001065>
- Ikhwan, A. Z. N., Napitupulu, T. P., Sumerta, I. N., Masrukhin, Kusmiati, Yuliani, Y., Sudiana, I. M., Idris, Kanti, A., & Lisdiyanti, P. (2023). Investigation of cellulolytic yeast from soil and leaf litter of savanna in Kupang, East Nusa Tenggara, Indonesia. *The First International Conference on Neuroscience and Learning Technology (ICONSATIN 2021)*, 2679, 050006. DOI: <https://doi.org/10.1063/5.0118636>
- Ilmi, M., Badrani, A., & Fauziyah, A. (2023). Increasing lipid production from *Zygosaccharomyces siamensis* AP1 in molasses substrate using sequencing batch method. *Preparative Biochemistry & Biotechnology*, 53(3), 288–296. DOI: <https://doi.org/10.1080/10826068.2022.2081859>
- Ilmi, M., & Siswontoro, M. (2021). Lipid Production from *Zygosaccharomyces siamensis* AP1 Using Glycerol as a Carbon Source. *Proceedings of the 10th International Seminar and 12th Congress of Indonesian Society for Microbiology (ISISM 2019)*, 15. DOI: <https://doi.org/10.2991/absr.k.210810.014>
- Jagtap, S. S., Liu, J.-J., Walukiewicz, H. E., Pangilinan, J., Lipzen, A., Ahrendt, S., Koriabine, M., Cobaugh, K., Salamov, A., Yoshinaga, Y., Ng, V., Daum, C., Grigoriev, I. V., Slininger, P. J., Dien, B. S., Jin, Y.-S., & Rao, C. V. (2022). Near-Complete Genome Sequence of *Zygosaccharomyces rouxii* NRRL Y-64007, a Yeast Capable of Growing on Lignocellulosic Hydrolysates. *Microbiology Resource Announcements*, 11(5), e0005022. DOI: <https://doi.org/10.1128/mra.00050-22>

- Ji, X., Chen, L., Yang, G., Huang, H., Hu, N., Jiang, Y., & Ni, J. (2024). Mutagenesis and fluorescence-activated cell sorting of oleaginous *Saccharomyces cerevisiae* and the multi-omics analysis of its high lipid accumulation mechanisms. *Bioresource Technology*, *406*, 131062. DOI: <https://doi.org/10.1016/j.biortech.2024.131062>
- Kanti, A. (2022). *Diversitas Khamir Indonesia untuk pengembangan Biofuel dan Bioindustri*. Penerbit BRIN. DOI: <https://doi.org/10.55981/brin.727>
- Kataya, A., Nascimento, J. R. S., Xu, C., Garneau, M. G., Koley, S., Kimberlin, A., Mukherjee, T., Mooney, B. P., Xu, D., Bates, P. D., Allen, D. K., Koo, A. J., & Thelen, J. J. (2025). Comparative Omics Reveals Unanticipated Metabolic Rearrangements in a High-Oil Mutant of Plastid Acetyl-CoA Carboxylase. *Journal of Proteome Research*, *24*(6), 2675–2688. DOI: <https://doi.org/10.1021/acs.jproteome.4c00947>
- Kerkhoven, E. J., Pomraning, K. R., Baker, S. E., & Nielsen, J. (2016). Regulation of amino-acid metabolism controls flux to lipid accumulation in *Yarrowia lipolytica*. *NPJ systems biology and applications*, *2*(1), 1-7. DOI: <https://doi.org/10.1038/npjbsba.2016.5>
- Lazar, Z., Liu, N., & Stephanopoulos, G. (2018). Holistic Approaches in Lipid Production by *Yarrowia lipolytica*. *Trends in Biotechnology*, *36*(11), 1157–1170. DOI: <https://doi.org/10.1016/j.tibtech.2018.06.007>
- Leber, C., Choi, J. W., Polson, B., & Da Silva, N. A. (2016). Disrupted short chain specific β -oxidation and improved synthase expression increase synthesis of short chain fatty acids in *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering*, *113*(4), 895–900. DOI: <https://doi.org/10.1002/bit.25839>
- Lee, S. Y., Weingarten, M., & Ottenheim, C. (2024). Current upstream and downstream process strategies for sustainable yeast lipid production. *Bioresource Technology*, *414*, 131601. DOI: <https://doi.org/10.1016/j.biortech.2024.131601>
- Lim, D. K. Y., Schuhmann, H., Sharma, K. K., & Schenk, P. M. (2015). Isolation of high-lipid *Tetraselmis suecica* strains following repeated UV-C mutagenesis, FACS, and high-throughput growth selection. *Bioenergy Research*, *8*(2), 750-759. DOI: <https://doi.org/10.1007/S12155-014-9553-2>
- Liu, S., Zhao, Y., Liu, L., Ao, X., Ma, L., Wu, M., & Ma, F. (2015). Improving cell growth and lipid accumulation in green microalgae *Chlorella* sp. via UV irradiation. *Applied Biochemistry and Biotechnology*, *175*(7), 3507-3518. DOI: <https://doi.org/10.1007/S12010-015-1521-6>
- Llamas, M., Magdalena, J. A., González-Fernández, C., & Tomás-Pejó, E. (2020). Volatile fatty acids as novel building blocks for oil-based chemistry via oleaginous yeast fermentation. *Biotechnology and Bioengineering*, *117*(1), 238–250. DOI: <https://doi.org/10.1002/bit.27180>
- Martinez-Silveira, A., Pereyra, V., Garmendia, G., Rufo, C., & Vero, S. (2019). Optimization of culture conditions of *Rhodotorula graminis* S1/2R to obtain saponifiable lipids for the production of second-generation biodiesel. *Environmental Sustainability*, *2*(4), 419–428. DOI: <https://doi.org/10.1007/s42398-019-00085-x>
- Ma, M., Han, P., Zhang, R., & Li, H. (2013). Ultrastructural changes of *Saccharomyces cerevisiae* in response to ethanol stress. *Canadian Journal of Microbiology*, *59*(9), 589–597. DOI: <https://doi.org/10.1139/cjm-2012-0745>
- Muthuraj, M., Selvaraj, B., Palabhanvi, B., Kumar, V., & Das, D. (2019). Enhanced lipid content in *Chlorella* sp. FC2 IITG via high energy irradiation mutagenesis. *Korean Journal of Chemical Engineering*, *36*(1), 63-70. DOI: <https://doi.org/10.1007/S11814-018-0180-Z>

- Nascimento, I. A., Marques, S. S., Cabanelas, I. T. D., Pereira, S. A., Druzian, J. I., de Souza, C. O., Vich, D. V., de Carvalho, G. C., & Nascimento, M. A. (2013). Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria. *Bioenergy Research*, 6(1), 1–13. DOI: <https://doi.org/10.1007/S12155-012-9222-2>
- Omura, S. (1976). The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. *Bacteriological Reviews*, 40(3), 681–697. DOI: <https://doi.org/10.1128/br.40.3.681-697.1976>
- Ono, T., Kesado, T., Awaya, J., & Omura, S. (1974). Target of inhibition by the anti-lipogenic antibiotic cerulenin of sterol synthesis in yeast. *Biochemical and Biophysical Research Communications*, 57(4), 1119–1124. DOI: [https://doi.org/10.1016/0006-291x\(74\)90812-2](https://doi.org/10.1016/0006-291x(74)90812-2)
- Qian, X., Zhou, X., Zhou, D., Zhou, J., Xin, F., Dong, W., Zhang, W., & Jiang, M. (2023). Biodiesel production from microbial lipids using oleaginous yeasts. In *Handbook of biofuels production* (pp. 199–229). Elsevier. DOI: <https://doi.org/10.1016/B978-0-323-91193-1.00007-X>
- Qiao, W., Dong, G., Xu, S., Li, L., & Shi, S. (2023). Engineering propionyl-CoA pools for de novo biosynthesis of odd-chain fatty acids in microbial cell factories. *Critical Reviews in Biotechnology*, 43(7), 1063–1072. DOI: <https://doi.org/10.1080/07388551.2022.2100736>
- Qin, N., Li, L., Wang, Z., & Shi, S. (2023). Microbial production of odd-chain fatty acids. *Biotechnology and Bioengineering*, 120(4), 917–931. DOI: <https://doi.org/10.1002/bit.28308>
- Raman, S., Rogers, J. K., Taylor, N. D., & Church, G. M. (2014). Evolution-guided optimization of biosynthetic pathways. *Proceedings of the National Academy of Sciences*, 111(50), 17803–17808. DOI: <https://doi.org/10.1073/pnas.1409523111>
- Ratledge, C. (2014). The role of malic enzyme as the provider of NADPH in oleaginous microorganisms: a reappraisal and unsolved problems. *Biotechnology Letters*, 36(8), 1557–1568. DOI: <https://doi.org/10.1007/s10529-014-1532-3>
- Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G., & Tredici, M. R. (2009). Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering*, 102(1), 100–112. DOI: <https://doi.org/10.1002/BIT.22033>
- Saha, S., Laha, D., Mandal, E., Datta, D., Das, B., & Jeon, B.-H. (2025). The potency of oleaginous yeast *Lipomyces starkeyi* in organic waste valorization to biodiesel. *Energy & Environment*, 36(5), 2131–2151. DOI: <https://doi.org/10.1177/0958305X241292285>
- Sajish, S., Singh, S., & Nain, L. (2022). Yeasts for Single Cell Oil Production from Non-conventional Bioresources. In J. K. Saini & R. K. Sani (Eds.), *Microbial biotechnology for renewable and sustainable energy* (pp. 337–364). Springer Nature Singapore. DOI: https://doi.org/10.1007/978-981-16-3852-7_13
- Salsabila, R., & Ilmi, M. (2021). Lipid production from *Zygosaccharomyces siamensis* AP1 using sequencing batch method with acetic acid as carbon source. *IOP Conference Series: Earth and Environmental Science*, 743(1), 012096. DOI: <https://doi.org/10.1088/1755-1315/743/1/012096>
- Samavi, M., & Rakshit, S. K. (2022). Value-added products from microbial lipid. In *Biomass, Biofuels, Biochemicals* (pp. 331–347). Elsevier. DOI: <https://doi.org/10.1016/B978-0-323-90631-9.00015-6>
- Santos, C. N. S., Xiao, W.-H., & Stephanopoulos, G. (2012). Rational, combinatorial, and

- genomic approaches for engineering L-tyrosine production in *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 109(34), 13538-13543. DOI: <https://doi.org/10.1073/pnas.1206346109>
- Saravanan, S., Kalanthoden, A. N., Karthikayan, R., Kavitha, M., & Rani, S. K. (2017). Improvement of Invertase Synthesis by the Mutant *Saccharomyces cerevisiae* through UV Mutagenesis. *Universal Journal of Microbiology Research*, 5(2), 17–24. DOI: <https://doi.org/10.13189/ujmr.2017.050201>
- Sekova, V., Bobrova, E., Isakova, E., & Deryabina, Yu. (2020). The Antioxidant Enzymes Activity From the Poly-extromophilic *Yarrowia lipolytica* Yeast Under Oxidative Stress During Long-lasting Cultivation. *Bulletin of Science and Practice*, 6(12), 23–35. DOI: <https://doi.org/10.33619/2414-2948/61/02>
- Shi, J., Feng, H., Lee, J., & Ning Chen, W. (2013). Comparative proteomics profile of lipid-cumululating oleaginous yeast: an iTRAQ-coupled 2-D LC-MS/MS analysis. *Plos One*, 8(12), e85532. DOI: <https://doi.org/10.1371/journal.pone.0085532>
- Sivaramakrishnan, R., & Incharoensakdi, A. (2023). UV mutagenesis followed by hydrogen peroxide treatment ameliorates lipid production and omega-3 fatty acids levels in *Chlorella* sp. *Algal Research*, 73, 103195. DOI: <https://doi.org/10.1016/j.algal.2023.103195>
- Soedarmodjo, T. P., Rachma, F. A., Aparamarta, H. W., & Widjaja, A. (2019). Study of UV-B Mutation Effect on pH Resistance and Lipid Production of Microalgae *Botryococcus braunii*. *Jurnal TANAH TROPIKA (Journal of Tropical Soils)*, 30(3), 101. DOI: <https://doi.org/10.12962/j20882033.v30i3.5475>
- Szczepańska, P., Hapeta, P., & Lazar, Z. (2022). Advances in production of high-value lipids by oleaginous yeasts. *Critical Reviews in Biotechnology*, 42(1), 1–22. DOI: <https://doi.org/10.1080/07388551.2021.1922353>
- Tanaka, A., Hagihara, T., Nishikawa, Y., Mishina, M., & Fukui, S. (1976). Effects of the anti-Lipogenic Antibiotic Cerulenin on Growth and Fatty Acid Composition of n-Alkane-Utilizing *Candida lipolytica*. *European Journal of Applied Microbiology*, 3(2), 115–124. DOI: <https://doi.org/10.1007/BF00928430>
- Tapia, E., Anschau, A., Coradini, A. L., T Franco, T., & Deckmann, A. C. (2012). Optimization of lipid production by the oleaginous yeast *Lipomyces starkeyi* by random mutagenesis coupled to cerulenin screening. *AMB Express*, 2(1), 64. DOI: <https://doi.org/10.1186/2191-0855-2-64>
- Teco-Bravo, J. I., Barahona-Pérez, L. F., Reyes-Sosa, C. F., López-Pacheco, I. Y., Barceló, D., Iqbal, H. M. N., & Parra-Saldívar, R. (2019). Enhanced production of triacylglycerols and polyunsaturated fatty acids in novel acid-tolerant mutants of the green microalga *Chlorella saccharophila*. *Bioprocess and Biosystems Engineering*, 42(10), 1613-1626. DOI: <https://doi.org/10.1007/S00449-019-02153-2>
- Tehlivets, O., Scheuringer, K., & Kohlwein, S. D. (2007). Fatty acid synthesis and elongation in yeast. *Biochimica et Biophysica Acta*, 1771(3), 255–270. DOI: <https://doi.org/10.1016/j.bbalip.2006.07.004>
- Tensingh, J. A. S., & Shankar, V. (2022). Sustainable production of biodiesel using UV mutagenesis as a strategy to enhance the lipid productivity in *R. mucilaginosa*. *Sustainability*, 14(15), 9079. DOI: <https://doi.org/10.3390/su14159079>

- Vardar-Yel, N., Yelboğa, E., Karagüler, N. G., & Çetin, D. (2024). Enhancement of docosahexaenoic acid production by UV mutagenesis coupled with flow cytometry screening in *Schizochytrium* sp. S31. *Caucasian Journal of Science*, 11(2), 153-169. DOI: <https://doi.org/10.48138/cjo.1559402>
- Vázquez, J., Grillitsch, K., Daum, G., Mas, A., Beltran, G., & Torija, M. J. (2019). The role of the membrane lipid composition in the oxidative stress tolerance of different wine yeasts. *Food Microbiology*, 78, 143–154. DOI: <https://doi.org/10.1016/j.fm.2018.10.001>
- Vello, V., Mohamed, R. M. S. R., Amin, M. N. M., Mubarak, N. A., Khalid, M., Abdullah, E. C., & Ibrahim, M. N. M. (2014). Lipid productivity and fatty acid composition-guided selection of *Chlorella* strains isolated from Malaysia for biodiesel production. *Journal of Applied Phycology*, 26(5), 1399–1413. DOI: <https://doi.org/10.1007/S10811-013-0160-Y>
- Wakil, S. J., Stoops, J. K., & Joshi, V. C. (1983). Fatty acid synthesis and its regulation. *Annual Review of Biochemistry*, 52, 537–579. DOI: <https://doi.org/10.1146/annurev.bi.52.070183.002541>
- Wang, J., Li, R., Lu, D., Ma, S., Yan, Y., & Li, W. (2009). A quick isolation method for mutants with high lipid yield in oleaginous yeast. *World Journal of Microbiology & Biotechnology*, 25(5), 921–925. DOI: <https://doi.org/10.1007/s11274-009-9960-2>
- Wang, Y., Zhang, S., Liu, H., Zhang, L., Yi, C., & Li, H. (2015). Changes and roles of membrane compositions in the adaptation of *Saccharomyces cerevisiae* to ethanol. *Journal of Basic Microbiology*, 55(12), 1417–1426. DOI: <https://doi.org/10.1002/jobm.201500300>
- Wen, J., Huang, L. L., Wang, D., Chen, Y., Geng, L., & Mao, X. (2025). UV mutagenesis enhances DHA biosynthesis in *Schizochytrium* sp. via metabolic reprogramming. *Biotechnology Journal*, 20(1), e70107. DOI: <https://doi.org/10.1002/biot.70107>
- Wusnah, W., Akbar, M. D., Supardan, M. D., Haryani, S., & Yunardi, Y. (2023). An overview of the potential utilisation of oleaginous yeast for biodiesel feedstock and wastewater treatment. *IOP Conference Series: Earth and Environmental Science*, 1182(1), 012018. DOI: <https://doi.org/10.1088/1755-1315/1182/1/012018>
- Wu, C.-C., Ohashi, T., Misaki, R., Limtong, S., & Fujiyama, K. (2020). Ethanol and H₂O₂ stresses enhance lipid production in an oleaginous *Rhodotorula toruloides* thermotolerant mutant L1-1. *FEMS Yeast Research*, 20(4). DOI: <https://doi.org/10.1093/femsyr/foaa030>
- Yamada, R., Kashiwara, T., & Ogino, H. (2017). Improvement of lipid production by the oleaginous yeast *Rhodospiridium toruloides* through UV mutagenesis. *World Journal of Microbiology & Biotechnology*, 33(5), 99. DOI: <https://doi.org/10.1007/s11274-017-2269-7>
- Yang, K.-M., Lee, N.-R., Woo, J.-M., Choi, W., Zimmermann, M., Blank, L. M., & Park, J.-B. (2012). Ethanol reduces mitochondrial membrane integrity and thereby impacts carbon metabolism of *Saccharomyces cerevisiae*. *FEMS Yeast Research*, 12(6), 675–684. DOI: <https://doi.org/10.1111/j.1567-1364.2012.00818.x>
- Zhang, H., Wu, C., Wu, Q., Dai, J., & Song, Y. (2016). Metabolic flux analysis of lipid biosynthesis in the yeast *Yarrowia lipolytica* using ¹³C-labeled glucose and gas chromatography-mass spectrometry. *PloS one*, 11(7), e0159187. DOI: <https://doi.org/10.1371/journal.pone.0159187>