Biosaintifika 17 (2) (2025): 249-259 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Valorization of Oil Palm Empty Fruit Bunch Waste Enhanced with Molasses for Erythritol Production

Jeffersen Hosea Setiabang[1], Michael Ryan Fildy[1], Kezia Zefanya Limawan[1], Advent Roan Widiyono[1], Nathanael Darrell Yonas[1], Jocelyn Nataniel[1,2], Indra Kurniawan Saputra[3,4], Riahna Kembaren[1*]

[1]Department of Biotechnology, School of Life Sciences, Indonesia International Institute for Life Sciences, Jakarta, Indonesia
[2]International Center for Biotechnology, Osaka University, Suita, Japan, 565-0871
[3]Department of Chemistry, University of Warwick, Coventry, United Kingdom
[4]Biotechnology program, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Malang, Indonesia

*Corresponding Author: riahna.kembaren@i3l.ac.id

Submitted: 2025-03-08. Revised: 2025-05-12. Accepted: 2025-07-16.

Abstract. Indonesia, a leading palm oil producer, generates 1,250 tons of Oil Palm Empty Fruit Bunches (OPEFB) waste daily, typically disposed of through polluting combustion. This study explores repurposing OPEFB as a substrate for erythritol production, a popular sweetener with minimal impact on blood sugar. The research utilized *Moniliella pollinis* mutant SP5, a yeast capable of producing erythritol from various substrates. The process involved acid hydrolysis of OPEFB followed by fermentation. When fortified with 15% v/v molasses, OPEFB successfully served as an optimal carbon source for erythritol production, achieving a mass yield of 0.2878 g/g. Further improvement came through the use of ethyl methane sulfonate (EMS) mutagenesis, with the best mutant (mutant 7) reaching a yield mass of 0.3298 g/g and volumetric productivity of 0.0534 g/L/h. This research aims to advance Indonesia's self-sufficiency in erythritol production while providing a sustainable solution for OPEFB waste reduction, simultaneously addressing economic and environmental concerns.

Keywords: Erythritol; Molasses; *Moniliella pollinis*; Mutagenesis; OPEFB

How to Cite: Setiabang, J. H., Fildy, M. R., Limawan, K. Z., Widiyono, A. R., Yonas, N. D., Nataniel, J., Saputra, I. K., & Kembaren, R. (2025). Valorization of Oil Palm Empty Fruit Bunch Waste Enhanced with Molasses for Erythritol Production. *Biosaintifika: Journal of Biology & Biology Education, 17(2)*, 249-259.

DOI: http://dx.doi.org/10.15294/biosaintifika.v17i2.22551

INTRODUCTION

Sweetness enhances food palatability by improving mouthfeel and masking unfavorable flavors, but high sugar consumption endangers diabetics (Asseo & Niv, 2022). With global diabetes cases projected to increase from 537 million in 2021 to 643 million in 2030, low-calorie sweetener demand continues rising (Soliman et al., 2024; Daher et al., 2022). Erythritol presents an ideal sucrose alternative, offering 60-70% of sucrose's sweetness with minimal calories (0.2 kcal/g) (Xu et al., 2022). Its antioxidant properties and non-diabetogenic, non-carcinogenic nature make it suitable for diabetics and individuals with obesity. Traditional commercial erythritol production methods are inefficient and costly (Soetan et al., 2023). Biotechnological approaches using microorganisms to convert inexpensive carbon sources provide more economical alternatives (Deshpande et al., 2022). Oil Palm Empty Fruit Bunch (OPEFB), a major palm oil industry waste, represents one such carbon source (Wen et al., 2022). Indonesia, which produced 83% of global palm oil exports in 2013, generates approximately 51.8 million tons of OPEFB annually (Indriati et al., 2021; Shigetomi et al., 2020).

Most OPEFB waste is currently incinerated, releasing pollutants and contributing to global warming (Adu et al., 2022). This is particularly unfortunate as OPEFB is enriched with sugars, including glucose, that could serve as an excellent substrate for bioprocessing (Rosli et al., 2017). Indonesia's abundant OPEFB resources and green economy initiatives create opportunities to repurpose this waste into valuable products using microorganisms like *Moniliella pollinis*. Research has shown that *M. pollinis* MUCL 40570 produces erythritol under high osmotic pressure conditions

(Hijosa-Valsero et al., 2022). While native strains yield relatively low erythritol amounts, chemical mutation can develop superior variants with improved efficiency.

This research enhances erythritol productivity using OPEFB by implementing chemical mutations and molasses supplementation with *M. pollinis* SP5. Molasses, a sugar-processing byproduct, contains nutrients essential for yeast growth, including carbon, nitrogen, vitamins, and minerals that serve as enzyme cofactors (Daza-Serna et al., 2021). Its approximately 50% sugar content, primarily sucrose, can be hydrolyzed by *M. pollinis* into glucose and fructose for growth and erythritol production (Sjölin et al., 2024). Molasses' high mineral salt content acts as a buffer system, maintaining a suitable pH (Mangwanda et al., 2023), while its vitamin content, particularly biotin and thiamine, supports yeast metabolism (Evers et al., 2023). Preliminary studies with *M. pollinis* SP5 achieved an erythritol yield of 0.120 g/g with volumetric productivity of 0.065 g/L/h.

The production of erythritol from OPEFB supplemented with molasses, optimized via chemical mutagenesis of *M. pollinis* SP5, presents a sustainable solution that supports food security, health independence, and aligns with UN Sustainable Development Goals centered on societal well-being. By utilizing underused agricultural waste, this study advances scientific knowledge, promotes circular economy practices, reduces environmental pollution, and fosters the commercialization of healthier sugar substitutes.

METHODS

Oil Palm Empty Fruit Bunch (OPEFB) Hydrolysate Preparation.

In the first stage of OPEFB processing, the OPEFB was chopped, dried, blended, and mixed into 2% (v/v) H_2SO_4 with a ratio of 1:10 (OPEFB: acid) in a sealed 1 L Duran bottle. Then, the Duran bottle was autoclaved using the *SX-700* Autoclave High-Pressure Steam Sterilizer for 1 hour at 121°C. The resulting hydrolysate was filtered through a cheesecloth into a 1 L beaker and neutralized with $Ca(OH)_2$ until pH 5.5, then vacuum filtered with 0.22 µm Whatman Filter Paper and a Buchner funnel. The neutralized hydrolysate was analyzed using High-Performance Liquid Chromatography (HPLC) to measure glucose, sucrose, arabinose, xylose, and fructose levels (Clarissa et al., 2024). To detoxify the OPEFB hydrolysate from fermentation inhibitors (e.g., furfural and guaiacol), 8.33 mM of sterile polyethylene glycol 6000 (PEG-6000) was added as much as 10% (v/v) of the hydrolysate that has been filtered using a 0.22 µm syringe filter (Nogueira et al., 2021).

Activation and Production of Erythritol Using *Moniliella pollinis* SP5

To activate the yeast, *M. pollinis* SP5 was inoculated into a 250 mL Erlenmeyer flask containing 100 mL of Potato Dextrose Broth (PDB) medium, then incubated at 28°C with 150 rpm agitation for 2 days (Kembaren et al., 2025). After incubation, the preculture concentration was adjusted to a concentration of 1×10^7 Colony Forming Unit per Milliliter (CFU/mL) and then transferred to 300 mL of OPEFB Hydrolyzed Yeast Minerals Media (OHYM) (OHYM composition is 10 g/L yeast extract, 2 g/L NH_4Cl, 1 g/L $MgSO_4\cdot7H_2O$, 0.2 g/L KH_2PO_4, and 0.07 g/L $ZnSO_4\cdot7H_2O$) production medium with a 10% (v/v) preculture ratio. Then, this was incubated at 28°C with 150 rpm agitation for 7 days. During the incubation period, daily observations were made on the cell culture based on cell count (CFU/mL) and pH using an *Ohaus pH meter*. The erythritol content in the cell culture supernatant was analyzed using HPLC (*Thermo Scientific UltiMate 3000*).

Optimization of Erythritol Production with the Addition of Molasses as a Supplementary Substrate

Optimization of erythritol production was carried out by adding molasses in 3 different concentrations of 15%, 25%, and 50% (v/v) in 300 mL of OHYM production media. Before the addition of 10% (v/v) preculture into the production media, 8.33 mM of sterile polyethylene glycol 6000 (PEG-6000) was added as much as 10% (v/v) of total media volume for detoxification. Then, the production media were incubated for 7 days at 28°C with 150 rpm agitation using an Incubator-Shaker (*TOU-50N Orbital Shaker Incubator*) (Kembaren et al., 2025). During the incubation period, daily observations were made on the cell culture based on cell count (CFU/mL) and pH (using a pH meter), which were all done with three technical replicates. The erythritol content in the cell culture supernatant was analyzed using HPLC.

$$X = \frac{Negative\ Control\ (CFU/mL) - Mutant\ Plate\ with\ Least\ Colony\ (CFU/mL)}{Negative\ Control\ (CFU/mL)} \times 100\% \quad (1)$$

Chemical Mutation in *Moniliella pollinis* Cultures

Mutations in *M. pollinis* SP5 were performed using a chemical mutagen, specifically ethyl methane sulfonate (EMS) (Khatape et al., 2023). The preculture for mutation was grown using 10 mL PDB media and incubated for 2 days at 28°C with 150 rpm agitation using an Incubator-Shaker (*TOU-50N Orbital Shaker Incubator*). Before mutation, the cell concentration was checked using the Miles-Misra method. The preculture concentration was adjusted to a concentration of 1×10^7 CFU/mL. The preculture was divided into 4 microtubes, with each microtube containing an aliquot of 1 mL preculture. The microtubes were then centrifuged to separate the cells from the growth medium. The pellet was washed using phosphate-buffered saline (PBS) twice and resuspended in 1 mL PBS. Sterile EMS was added to the 1 mL suspension with concentrations of 0%, 1%, 2%, and 3% (v/v) separately in each microtube, then incubated for one hour at room temperature in a dark condition. After incubation, 8 mL of 10% sterile sodium thiosulfate was added to each microtube and incubated at 28°C for 3 hours in the dark condition. As a negative control for the experiment, the same procedures were repeated without the addition of EMS. From these samples, 20 µL from each microtube of mutation result suspension was then spread on Potato Dextrose Agar (PDA) medium and then incubated for 4 days at room temperature. The calculation of the mortality rate of the mutant colonies was performed after the incubation period. The mortality rate (X) was calculated using equation (1).

For mutant screening, ten growing mutant colonies on the PDA medium were selected randomly to be grown in the OHYM medium fortified with 15% (v/v) molasses for 5 days at 28°C and 150 RPM agitation. The negative control (untreated EMS) colony will also be kept under the same growth conditions for comparison. The erythritol content in the cell culture supernatant was analyzed using HPLC. The two mutants with the highest erythritol production were cultured within the OHYM medium fortified with 15% (v/v) molasses for 7 days at 28°C with 150 rpm agitation. During incubation, daily observations were made on the cell culture based on cell count (CFU/mL) and pH (using a pH meter), which were all done with three technical replicates. The erythritol content in the cell culture supernatant was analyzed using HPLC.

Analysis of Erythritol Production and Substrate Consumption Using HPLC

The supernatant of the collected cell culture was analyzed for erythritol content and monosaccharide content using standards such as glucose (*Merck*), sucrose (*Sigma-Aldrich*), arabinose (*VWR*), xylose (*Sigma-Aldrich*), fructose (*Merck*), and meso-erythritol (*TCI*) for identification and quantification using HPLC using a *Thermo Scientific Ultimate 3000 HPLC* with a *Shodex™ SUGAR SP0810* column (8.0 mm I.D. x 300 mm). HPLC analysis was run with the following settings: column temperature at 80°C, mobile phase with 100% type 1 distilled water, Refractor Index (RI) detector at 50°C, flow rate at 0.7 mL/min, and run time of each sample for 35 minutes (Kembaren et al., 2025).

The yield mass and the volumetric productivity were calculated based on the HPLC results. The calculation for erythritol yield mass (Y) was done using equation (2)

$$Y = \frac{erythritol\ concentration\ (g/L)}{total\ consumed\ carbon\ (g/L)} \quad (2)$$

The calculation for erythritol volumetric productivity (V) was done using equation (3)

$$V = \frac{erythritol\ concentration\ (g/L)}{duration\ of\ incubation\ (h)} \quad (3)$$

RESULTS AND DISCUSSION

pH Results Analysis

Microorganisms in bioprocesses require specific pH ranges for optimal growth, with deviations potentially impairing metabolism and affecting yield. Monitoring culture pH provides insight into *Moniliella pollinis* growth patterns, where decreasing pH indicates organic acid accumulation from metabolic activity, confirming fermentation progress (Pau et al., 2022). However, extreme pH decreases negatively impact erythritol production by reducing viable cells, while significant pH increases may signal cell death and fermentation cessation (Carsanba et al., 2021). Analysis of *M. pollinis* SP5 strain cultured for 7 days revealed that OPEFB media without

molasses exhibited more dramatic pH fluctuations compared to molasses-supplemented media. Molasses functions as an alternative carbon source, containing 30-35% (w/w) sucrose, 10-25% (w/w) glucose, 10-25% (w/w) fructose, plus essential minerals and vitamins that optimize cell growth periods (Jamir et al., 2021). Figure 1 demonstrates that unsupplemented media showed increasing pH from day 5, peaking at day 7, indicating cell death as ammonia accumulated. Conversely, molasses-supplemented media maintained relatively stable pH, showing only slight increases on day 5, suggesting continued growth and stationary phase maintenance. This stability stems from molasses' inherent buffer capacity, which helps maintain media pH equilibrium (Ungureanu et al., 2022). These findings highlight molasses' importance in maintaining favorable conditions for extended *M. pollinis* cultivation and erythritol production.

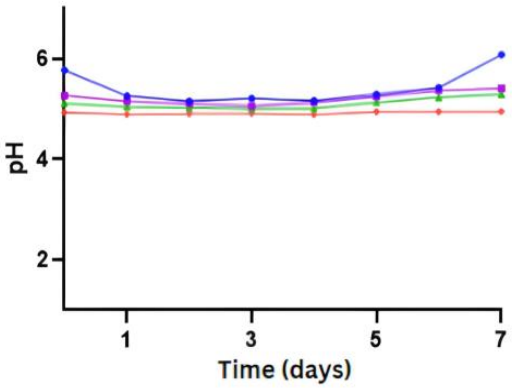

Figure 1. pH measurement result of *M. pollinis* SP5 strain for a period of 7 days. (Symbols: ●: OPEFB + 0% (v/v) molasses, ■: OPEFB + 15% (v/v) molasses, ▲: OPEFB + 25% (v/v) molasses, and ◆: OPEFB + 50% (v/v) molasses).

Correlation Analysis of Viable Cell Count (CFU/mL) and Sugar Content

The Miles-Misra method was employed to determine viable cell counts in production media by plating diluted culture samples to produce countable colonies. This method enables estimation of cell density at specific timepoints, as CFU/mL correlates directly with living colonies in the media. Measuring CFU/mL at intervals reveals growth curves showing cell lifecycle phases: lag (adaptation), exponential (growth), stationary (balanced growth and death), and death phase (declining cell numbers) (Ram et al., 2019). Figure 2 reveals that media with higher molasses concentrations (25% and 50% v/v) contain significantly lower detectable levels of xylose and arabinose compared to lower molasses formulations (0% and 15% v/v). This occurs because sucrose peaks from molasses overshadow xylose and arabinose peaks in HPLC readings. While OPEFB hydrolysate primarily yields xylose and arabinose, molasses predominantly contains sucrose and glucose (Mardawati et al., 2017). The substantial concentration differences hinder detection of the lower-concentration sugars during HPLC analysis.

OPEFB without molasses showed only a brief exponential phase on day one, followed by a significant decline. HPLC analysis revealed xylose and arabinose as the main sugars from OPEFB hydrolysis, but their minimal decrease over time indicates OPEFB alone provides suboptimal support for *M. pollinis* growth. Conversely, OPEFB with molasses additions (15%, 25%, and 50% v/v) demonstrated more stable growth with extended exponential phases (days 1-3) and gradual declines. Media with 15% and 25% molasses showed gradual decreases in cell numbers during days 3-5. The 15% molasses formulation provided sufficient sugar for culture development while leaving minimal residual glucose and sucrose, evidenced by their significant decrease as fermentation progressed. Decreasing sucrose and glucose levels alongside increasing fructose and erythritol concentrations demonstrate the culture's substrate utilization and erythritol production capabilities. *M. pollinis* contains sucrose invertase enzyme, which hydrolyzes sucrose into glucose and fructose, with activity increasing as glucose levels decline (Erian & Sauer, 2022). This explains decreased sucrose levels by day 3 across all molasses-containing media, increased fructose, and relatively stable glucose levels due to simultaneous consumption and replenishment from sucrose.

A consistent spike in erythritol concentration appeared on day 3 in 15% and 25% molasses formulations, suggesting a correlation between invertase activity and erythritol production during the stationary phase. Subsequent glucose decreases likely resulted from rapid consumption for growth and erythritol production. Higher molasses concentrations, as shown in Figure 2 with 50% molasses addition, did not correlate with increased erythritol production. Hyperosmotic conditions triggered by high molasses concentrations cause adaptive cellular responses, redirecting carbon flux from erythritol production to cell growth for survival (Carly & Fickers, 2018; Yang et al., 2015). Between days 5-7, *M. pollinis*

SP5 cultures showed decreasing viable cell numbers, indicating the onset of the death phase. Therefore, 15% (v/v) molasses supplementation proved optimal for OPEFB media, achieving the best balance between providing sufficient substrate for growth and erythritol production while minimizing hyperosmotic stress effects seen at higher concentrations. This formulation supported extended exponential growth, efficient sucrose and glucose utilization, and consistent erythritol production, whereas higher concentrations redirected carbon flux toward cell survival rather than erythritol synthesis, reducing overall yield.

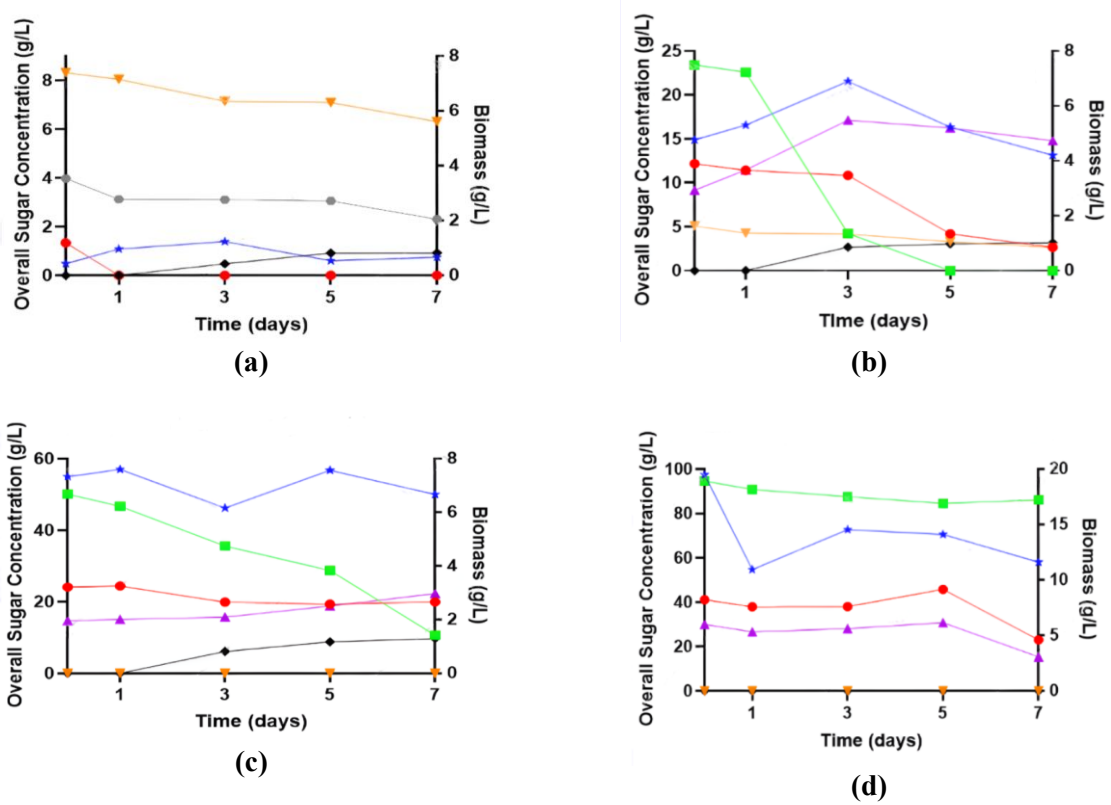

Figure 2. Correlation of viable colonies with erythritol production and carbon sources consumption in OPEFB production media: (a) without molasses, and with molasses at concentrations of (b) 15% (v/v), (c) 25% (v/v), and (d) 50% (v/v). (Symbols: ★: log CFU/mL, ●: glucose, ■: sucrose, ▲: fructose, ▼: xylose, ◆: erythritol, and ⬣: arabinose).

Table 1. *M. pollinis* SP5 productivity in erythritol production

Sample Media	Erythritol Concentration (g/L)	Erythritol Yield Mass (g/g)	Erythritol Volumetric Productivity (g/L/h)
OPEFB + 0% (v/v) molasses	0.9292	0.1854	0.0055
OPEFB + 15% (v/v) molasses	6.2421	0.2878	0.0372
OPEFB + 25% (v/v) molasses	9.7164	0.1753	0.0578
OPEFB + 50% (v/v) molasses	ND	NC	NC

*ND = Not Detected; NC = Non-Calculable

Yield Mass and Volumetric Productivity of Erythritol Production

Yield mass and volumetric productivity are essential metrics in bioproduct production as they directly indicate the efficiency, scalability, and economic viability of the process. Yield mass represents the effectiveness of converting substrates into the desired product, while volumetric productivity quantifies the production rate. Together, these metrics help assess the overall performance and profitability of the production process.

In the production of erythritol, OPEFB with the addition of 15% (v/v) molasses showed the highest mass yield of 0.2878 g/g. However, its volumetric productivity of erythritol or the rate of erythritol production is relatively lower than OPEFB with 25% (v/v) molasses addition (Table 1). This is due to the higher osmotic pressure of the media caused by the salts from the neutralization of OPEFB, where the cell response to osmotic pressure increases the yield of erythritol (Rywińska et al., 2024). A previous study by Khatape et al. in 2023 reported that the yield mass of erythritol produced by wild-type *M. pollinis* CBS 461.67 was 0.262 g/g on glucose media and similar growing conditions, so the production of erythritol using OPEFB media with 15% (v/v) molasses has proven to be quite effective.

Ethyl Methanesulfonate (EMS) Mutagenesis Result

Ethyl Methanesulfonate (EMS) is a chemical mutagen that induces random point mutations in the DNA of organisms, generating a diverse population of mutants. This genetic diversity increases the chances of discovering strains with desirable traits, such as enhanced bioproduct production, improved tolerance to environmental stresses, and resistance to inhibitors. EMS mutagenesis can also optimize specific traits, such as enzyme activity (Pantaya et al., 2024). Strains with enhanced enzyme activity can achieve faster and more efficient conversion of substrate into the target bioproduct (Luo et al., 2021). While some mutations may be beneficial, many others can be detrimental. The unpredictable EMS mutation can lead to undesirable traits, such as reduced growth rates, impaired metabolism, or decreased viability of the organism (Wang et al., 2022). Due to the random nature of EMS-induced mutations, a large number of mutants need to be screened to identify those with the desired traits (Cheng et al., 2018).

From the mutation results of *Moniliella pollinis* SP5 using ethyl methane sulfonate (EMS) in concentrations of 1% (v/v), 2% (v/v), and 3% (v/v), it can be seen that viable cells mutated using high concentrations of EMS have relatively low growth (Figure 3). Compared to the negative control, the 2% (v/v) mutation had the highest mortality rate of 99.38%, while the mortality rate of the 1% (v/v) mutation was not calculated due to colony count resulting in Too Numerous To Count (TNTC). For the screening stage, colonies mutated with 2% (v/v) EMS were used for the screening step. A mutation with 3% (v/v) EMS concentration had no growing colonies due to mutations in DNA occurring in essential genes for a culture's survival, causing the culture to not be able to function normally. A previous study by Khatape et al. in 2023 reported that the wild-type *M. pollinis* CBS 461.67 can still grow after being exposed to EMS at concentrations up to 5% (v/v). However, in this experiment, cultures exposed to 3% (v/v) EMS concentration showed no sign of growth. This is likely due to the different initial cell counts used for mutation in their study (3×10^8 cells/mL) (Khatape et al., 2023).

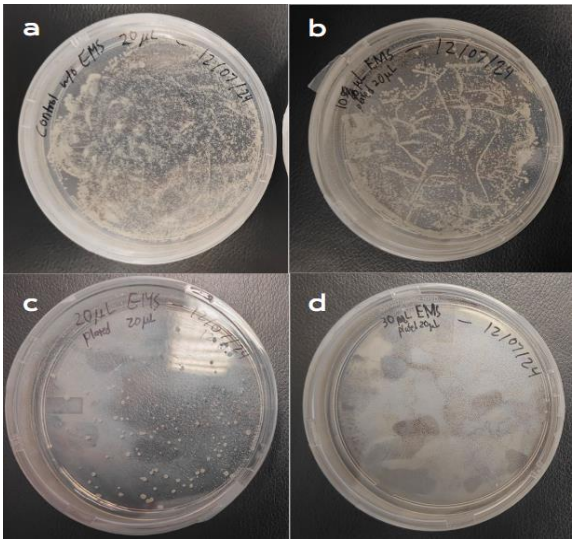

Figure 3. Comparison of M. pollinis SP5 colonygrowth across different EMS concentrations (a) control without EMS, (b) 1% (v/v) EMS, (c) 2% (v/v) EMS, and (d) 3% (v/v) EMS.

To find the highest erythritol-producing mutants, ten random surviving colonies were analyzed for their erythritol production after being grown for 5 days on OHYM media with 15% (v/v) molasses. *M. pollinis* SP5 (control) was also grown in the same conditions for comparison. Data obtained from mutant screening results were CFU/mL and the erythritol content of each

screening medium (Table 2). Mutants 7 and 10 achieved the highest erythritol concentrations, producing 6.19 g/L and 6.49 g/L, respectively, representing increases of 1.23-fold and 1.29-fold compared to the control culture (5.04 g/L). These enhancements are likely due to EMS-induced mutations that improved specific aspects of the erythritol biosynthesis pathway, either by enhancing enzyme activity or optimizing substrate utilization (Mirończuk et al., 2017). Based on the CFU/mL data of mutants 7 and 10, when compared to other mutants, the cell growth rate does not significantly affect the amount of erythritol produced because other mutants also had similar CFU/mL values, even mutant 8, with relatively high CFU/mL, had the least erythritol produced. Therefore, the tendency of the culture to produce erythritol was selected as the main criterion for choosing the best mutants. For that reason, both mutant 7 and 10 cultures were selected and were grown on a production medium of OHYM enhanced with 15% (v/v) molasses.

Table 2. Erythritol concentration and number of living cells in screened mutants and controls

Mutant Isolates	Erythritol Concentration (g/L)	CFU/mL	Erythritol Concentration Significance
1	5.54	5.5046	Increase**
2	5.74	5.5073	Increase**
3	5.46	5.5306	Increase**
4	5.41	5.3286	Increase**
5	4.96	5.1324	Decrease**
6	5.98	5.3795	Increase**
7	6.19	5.4251	Increase**
8	4.20	5.3713	Decrease**
9	5.29	5.2825	Increase**
10	6.49	5.3138	Increase**
Control (*M. pollinis* SP5)	5.04	5.6655	Control

Note: symbol *: p value <0.05 (significant); **: p value <0.01 (highly significant)

Based on the following graph (Figure 4), the pH between the control *M. pollinis* culture with mutants 7 and 10 showed a similar pH growth graph, where the stationary phase continued until day 7. This shows that the addition of 15% (v/v) molasses to OHYM media had a buffer capacity effect, which results in a more stable pH, indicating that culture growth continues.

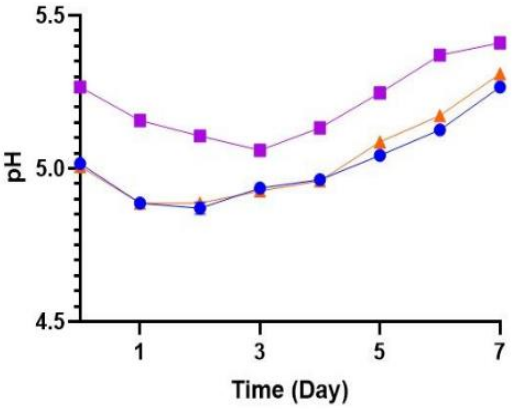

Figure 4. pH measurement results of *M. pollinis* mutant strain culture for 7 days. (Symbol explanation: ■: Control, ●: Mutant 7, ▲: Mutant 10).

As shown in Figure 5, the number of live cells in both mutants 7 and 10 had a similar value of log CFU/mL, and when compared to the control culture (Figure 2), the number of viable cells also reached a similar value of 6 log CFU/mL. This correlation indicates that the cell growth capabilities of mutant cultures 7 and 10 are not compromised by the mutagenesis. In addition, the viable cell graphs of mutants 7 and 10, when compared to the control culture, experienced an increase in cell number that lasted 1 day after inoculation, namely an exponential phase and a stationary phase that occurred earlier and lasted longer, from day 1 to 7. The following extension of the stationary phase gives more time for the cells to produce erythritol, and the rapid attainment of the stationary phase can increase the efficiency of sugar utilization into erythritol as well.

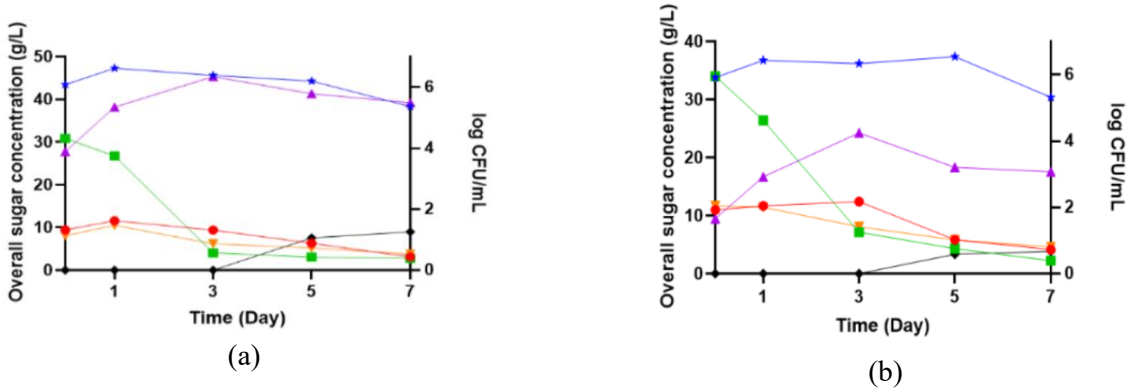

Figure 5. Number of viable mutant colonies on production media for 7 days for (a) mutant 7 and (b) mutant 10. (Explanation of symbols: ★: log CFU/mL, ●: glucose, ■: sucrose, ▲: fructose, ▼: xylose, and ◆: erythritol).

Based on the sugar data obtained through HPLC results, the mass yield and volumetric productivity were calculated to analyze the erythritol production capacity of both mutants (Table 3). The volumetric productivity of the two mutants, compared to the control in the same OHYM medium with 15% (v/v) molasses, increased significantly from 0.0372 g/L/h to 0.0534 g/L/h for mutant 7 and 0.0585 g/L/h for mutant 10. This indicates a faster erythritol production rate in the mutants. The yield mass of mutant 7 significantly increased from 0.2878 g/g in the control culture *M. pollinis* SP5 to 0.3298 g/g, demonstrating that mutant 7 has a higher sugar utilization efficiency for erythritol production. The data indicate that both mutants, 7 and 10, exhibit faster and higher erythritol production capacities. Notably, mutant 7 shows the highest conversion efficiency of sugar to erythritol. Mutants 7 and 10 were chosen as the samples with the highest potential because of their increased concentration and volumetric productivity compared to the control, which was cultivated in the same conditions. Especially mutant 7, which has the highest yield mass.

Table 3. Erythritol production productivity of *M. pollinis* mutant 7 and mutant 10.

Sample	Erythritol Concentration (g/L)	Yield Mass (g/g)	Volumetric Productivity (g/L/h)
Mutant 7	8.9732	0.3298	0.0534
Mutant 10	9.8289	0.2612	0.0585
Control	6.2421	0.2878	0.0372

Based on the results, OPEFB has been successfully utilized as the primary carbon source for erythritol production using *Moniliella pollinis* SP5 culture. Supplementing the medium with molasses further increased erythritol yield. Additionally, mutations induced by EMS treatment in *Moniliella pollinis* SP5, particularly in mutants 7 and 10, demonstrated significant potential to enhance erythritol production from waste in Indonesia. These mutants not only produced higher erythritol concentrations compared to the control but also maintained the stationary phase for a longer duration. With further optimization, these two mutants could boost erythritol productivity on an industrial scale, contributing to both sustainable production and a reduction in OPEFB waste burning in Indonesia.

This study demonstrates the potential of combining OPEFB and molasses as effective feedstocks for erythritol production- an approach not previously explored (Kembaren et al., 2025). In addition, it investigates the use of chemical mutagenesis to enhance microbial productivity, setting it apart from most existing research. By confirming the viability of these substrates, the study advances scientific understanding while contributing to societal progress through the promotion of a circular economy, stimulation of sustainable economic growth, and reduction of environmental pollution from improper OPEFB disposal.

CONCLUSION

Fermentation using the hydrolysate of Empty Palm Oil Bunches (OPEFB) as the main substrate by *Moniliella pollinis* SP5 over 7 days successfully produced erythritol. The addition of

15% (v/v) molasses to the production medium significantly increased erythritol yield, achieving a high mass yield of 0.2878 g/g. However, excessive molasses can reduce productivity due to increased osmotic pressure, emphasizing the need for optimal molasses concentration. EMS-induced mutations led to the development of two superior mutants, 7 and 10, which produced 1.23 and 1.29 times more erythritol than the control, with mutant 7 achieving a mass yield of 0.3298 g/g and volumetric productivity of 0.0534 g/L/h. This research has the potential to advance Indonesia's self-sufficiency in erythritol production while reducing OPEFB waste. Future work should focus on optimizing media components such as micronutrients, pH, and detoxification methods, as well as exploring additional mutagens or screening techniques to further enhance mutant quality.

ACKNOWLEDGEMENTS

We would like to express our deepest gratitude to the Ministry of Education, Culture, Research, and Technology of Indonesia (Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi Republik Indonesia), *Belmawa,* and *Program Kreativitas Mahasiswa* (PKM) 2024, who have given us the opportunity and funding to carry out this research in 2024.

AUTHOR CONTRIBUTION STATEMENT

Every author contributed significantly to the development of this manuscript. JHS, MRF, KZL, ARW, and NDY designed the study, conducted the experiment and data collection, data analysis, and manuscript writing under the supervision of RK and JN. RK, JN, and IKS proofread and provided substantial feedback regarding the manuscript draft before submission. All authors have read and approved the final manuscript and are accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

REFERENCES

Adu, M. O., Atia, K., Arthur, E., Asare, P. A., Obour, P. B., Danso, E. O., Frimpong, K. A., Sanleri, K. A., Asare-Larbi, S., Adjei, R., Mensah, G., & Andersen, M. N. (2022). The use of oil palm empty fruit bunches as a soil amendment to improve the growth and yield of crops. A meta-analysis. *Agronomy for Sustainable Development*, *42*(2). https://doi.org/10.1007/s13593-022-00753-z

Asseo, K., & Niv, M. Y. (2022). Harnessing food product reviews for personalizing sweetness levels. Foods, 11(13), 1872. https://doi.org/10.3390/foods11131872

Carly, F., & Fickers, P. (2018). Erythritol production by yeasts: a snapshot of current knowledge. *Yeast*, *35*(7), 455–463. https://doi.org/10.1002/yea.3306

Carsanba, E., Pintado, M., & Oliveira, C. (2021). Fermentation strategies for production of pharmaceutical terpenoids in engineered yeast. *Pharmaceuticals*, *14*(4), 295. https://doi.org/10.3390/ph14040295

Cheng, H., Wang, S., Bilal, M., Ge, X., Zhang, C., Fickers, P., & Cheng, H. (2018). Identification, characterization of two NADPH-dependent erythrose reductases in the yeast Yarrowia lipolytica and improvement of erythritol productivity using metabolic engineering. *Microbial Cell Factories*, *17*(1). https://doi.org/10.1186/s12934-018-0982-z

Clarissa, C., Valerie, L., Hadi, P., Purnamadjaja, A. M., Valentino, F., & Kembaren, R. (2024). Utilization Of Oil Palm Empty Fruit Bunches Enhanced With Molasses For Xylitol Production. *Jurnal Bioteknologi Dan Biosains Indonesia, 11*(2), 376–387. https://doi.org/10.11594/jbbi.11.2.7739

Daher, M., Fahd, C., Nour, A. A., & Sacre, Y. (2022). Trends and amounts of consumption of low-calorie sweeteners: A cross-sectional study. Clinical Nutrition ESPEN, 48, 427–433. https://doi.org/10.1016/j.clnesp.2022.01.006

Daza-Serna, L., Serna-Loaiza, S., Masi, A., Mach, R. L., Mach-Aigner, A. R., & Friedl, A. (2021). From the culture broth to the erythritol crystals: an opportunity for circular economy. Applied Microbiology and Biotechnology, 105(11), 4467–4486. https://doi.org/10.1007/s00253-

021-11355-2

Deshpande, M. S., Kulkarni, P. P., Kumbhar, Pramod S, & Ghosalkar, Anand R. (2022). Erythritol production from sugar-based feedstocks by Moniliella pollinis using lysate of recycled cells as nutrient source. Process Biochemistry, 112, 45–52. https://doi.org/10.1016/j.procbio.2021.11.020

Erian, A. M., & Sauer, M. (2022). Utilizing yeasts for the conversion of renewable feedstocks to sugar alcohols - a review. *Bioresource Technology*, *346*, 126296. https://doi.org/10.1016/j.biortech.2021.126296

Evers, M. S., Roullier-Gall, C., Morge, C., Sparrow, C., Gobert, A., Vichi, S., & Alexandre, H. (2023). Thiamine and biotin: Relevance in the production of volatile and non-volatile compounds during *Saccharomyces cerevisiae* alcoholic fermentation in synthetic grape must. *Foods*, *12*(5), 972. https://doi.org/10.3390/foods12050972

Hijosa-Valsero, M., Paniagua-García, A. I., & Díez-Antolínez, R. (2022). Cell immobilization for erythritol production. *Journal of Fungi*, *8*(12), 1286. https://doi.org/10.3390/jof8121286

Indriati, L., Elyani, N., & Dina, S. F. (2021). Empty fruit bunches, potential fiber source for Indonesian pulp and paper industry. *IOP Conference Series: Materials Science and Engineering*, *980*, 012045. https://doi.org/10.1088/1757-899x/980/1/012045

Jamir, L., Kumar, V., Kaur, J., Kumar, S., & Singh, H. (2021). Composition, valorization and therapeutical potential of molasses: a critical review. *Environmental Technology Reviews*, *10*(1), 131–142. https://doi.org/10.1080/21622515.2021.1892203

Kembaren, R., Parikesit, A. A., Nataniel, J., Dharmawan, N. A., Dungus, C. M., Angelique, P., & Aslanzadeh, S. (2025). Optimization of erythritol production through fermentation using molasses as carbon source. *Acta Biochimica Polonica, 71*. https://doi.org/10.3389/abp.2024.14000

Khatape, A. B., Rangaswamy, V., & Dastager, S. G. (2023). Strain improvement for enhanced erythritol production by Moniliella pollinis Mutant-58 using jaggery as a cost-effective substrate. *International Microbiology*. https://doi.org/10.1007/s10123-023-00411-8

Luo, J.-M., Zhu, W.-C., Cao, S.-T., Lu, Z.-Y., Zhang, M.-H., Song, B., Shen, Y.-B., & Wang, M. (2021). Improving biotransformation efficiency of Arthrobacter simplex by enhancement of cell stress tolerance and enzyme activity. Journal of Agricultural and Food Chemistry, 69(2), 704–716. https://doi.org/10.1021/acs.jafc.0c06592

Mirończuk, A. M., Biegalska, A., & Dobrowolski, A. (2017). Functional overexpression of genes involved in erythritol synthesis in the yeast Yarrowia lipolytica. *Biotechnology for Biofuels, 10*(1). https://doi.org/10.1186/s13068-017-0772-6

Nogueira, C. da C., Padilha, C. E. de A., & Santos, E. S. dos. (2021). Boosting second-generation ethanol titers from green coconut fiber by using high-concentration polyethylene glycol. Industrial Crops and Products, 166, 113494. https://doi.org/10.1016/j.indcrop.2021.113494

Pantaya, D., Alfriani, C. N. E., Asrianto, N., & Subagja, H. (2024). Effectiveness mutagenesis of bacillus subtilis using EMS (ethyl methane sulfonate) to increase xylanase enzyme activity. IOP Conference Series Earth and Environmental Science, 1338(1), 012022–012022. https://doi.org/10.1088/1755-1315/1338/1/012022

Ram, Y., Dellus-Gur, E., Bibi, M., Karkare, K., Obolski, U., Feldman, M. W., Cooper, T. F., Berman, J., & Hadany, L. (2019). Predicting microbial growth in a mixed culture from growth curve data. *Proceedings of the National Academy of Sciences*, *116*(29), 14698–14707. https://doi.org/10.1073/pnas.1902217116

Rosli , N. S., Harun, S., Jahim, J. M., & Othaman, R. (2017). Chemical and physical characterization of oil palm empty fruit bunch. *Malaysian Journal of Analytical Sciences, 21*(1), 188–196. http://dx.doi.org/10.17576/mjas-2017-2101-22

Rywińska, A., Tomaszewska-Hetman, L., Juszczyk, P., Rakicka-Pustułka, M., Bogusz, A., & Rymowicz, W. (2024). Enhanced Production of Erythritol from Glucose by the Newly Obtained UV Mutant Yarrowia lipolytica K1UV15. Molecules, 29(10), 2187–2187. https://doi.org/10.3390/molecules29102187

Shigetomi, Y., Ishimura, Y., & Yamamoto, Y. (2020). Trends in global dependency on the Indonesian palm oil and resultant environmental impacts. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-77458-4

Sjölin, M., Djärf, M., Ismail, M., Schagerlöf, H., Wallberg, O., Hatti-Kaul, R., & Sayed, M. (2024). Investigating the inhibitory factors of sucrose hydrolysis in sugar beet molasses with yeast and invertase. *Catalysts*, *14*(5), 330. https://doi.org/10.3390/catal14050330

Soetan, O. A., Ajao, F. O., & Ajayi, A. F. (2023).

Blood glucose lowering and anti-oxidant potential of erythritol: An in vitro and in vivo study. Journal of Diabetes and Metabolic Disorders, 22(2), 1217–1229. https://doi.org/10.1007/s40200-023-01237-x

Soliman, A. R., Hegazy, M., Ahmed, R. M., Abdelghaffar, S., Gomaa, M., Alwakil, S., Soliman, D., Sedky, L., & Shaltout, I. (2024). Dietary recommendations for people with diabetes in special situations: a position statement report by Arabic Association for the Study of Diabetes and metabolism (AASD). *Journal of Health, Population and Nutrition*, *43*(1). https://doi.org/10.1186/s41043-024-00619-y

Ungureanu, N., Vlăduț, V., & Biriș, S.-Ș. (2022). Sustainable valorization of waste and by-products from sugarcane processing. *Sustainability*, *14*(17), 11089. https://doi.org/10.3390/su141711089

Wang, C., Liu, B., Chen, M., Ning, J., Lu, X., & Wang, C. (2022). Mutations in growth-related genes induced by EMS treatment in scallops. Frontiers in Genetics, 13. https://doi.org/10.3389/fgene.2022.879844

Wen, A. L. K., Chew, J. J., Yiin, C. L., & Serene, S. M. L. (2022). Thermal degradation behavior and kinetic modeling of green solvents-delignified biomass: a sustainable biomass-to-energy approach. *Elsevier EBooks*, 89–103. https://doi.org/10.1016/b978-0-12-824388-6.00012-9

Xu, Y., Wu, Y., Liu, Y., Li, J., Du, G., Chen, J., Lv, X., & Liu, L. (2022). Sustainable bioproduction of natural sugar substitutes: Strategies and challenges. *Trends in Food Science & Technology*, *129*, 512–527. https://doi.org/10.1016/j.tifs.2022.11.008

Yang, L.-B., Dai, X.-M., Zheng, Z., Zhu, L., Zhan, X.-B., & Chi Chung Lin. (2015). Proteomic Analysis of Erythritol-Producing Yarrowia lipolytica from Glycerol in Response to Osmotic Pressure. *Journal of Microbiology and Biotechnology, 25*(7), 1056–1069. https://doi.org/ 10.4014/jmb.1412.12026