https://doi.org/10.48047/AFJBS.6.15.2024.7499-7510

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Erythritol production from crude glycerol by *Yarrowia lipolytica*

Lakshmi Pillai and Anita Katkade

Institute of Biological Science, Sage University, Indore, India
Corresponding author: *anukatkade88@gmail.com*

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.7499-7510

Abstract
Erythritol, a low-calorie sugar alcohol, has significant applications in the food and pharmaceutical industries. Traditionally produced through chemical synthesis, erythritol can also be obtained via biotechnological methods using microorganisms, offering a more sustainable alternative. In this study, erythritol production from crude glycerol was investigated using four strains of *Yarrowia lipolytica* (MTCC 9518, MTCC 9519, MTCC 9520, and MTCC 9521). The production of erythritol and mannitol, along with cellular growth and glycerol consumption, were monitored over seven days. Among the strains tested, *Y. lipolytica* MTCC 9519 demonstrated the highest erythritol concentration (56 $g \cdot L^{-1}$), yield (42% w/w), and productivity (0.37 $g \cdot L^{-1} \cdot h^{-1}$). In contrast, strains MTCC 9518 and MTCC 9520 produced lower concentrations of erythritol (23 and 29 $g \cdot L^{-1}$, respectively), resulting in lower yields (38% w/w) and productivity (0.15 and 0.11 $g \cdot L^{-1} \cdot h^{-1}$). The findings indicate that *Y. lipolytica* MTCC 9519 is the most suitable strain for erythritol production under the conditions tested.

Keywords: Glycerol, Erythritol, *Yarrowia lipolytica*, Fermentation and Optimization

Introduction

Glycerol is in abundant supply on the market because of rising biodiesel demand. Glycerol's value has a lot to give in terms of reducing the price of biodiesel manufacturing and decreasing environmental issues brought on by this bio fuel's manufacturers. The most significant organic acid produced during fermentation is citric

acid, which finds extensive usage in the food, drug, & chemical industries. Worldwide production of citric acid (CA) for industrial usage exceeds 2 million tonnes annually.

Erythritol is a naturally occurring sugar alcohol used as a low-calorie sweetener in various food and pharmaceutical products. Its advantages include a high digestive tolerance, no glycemic response, and a similar taste profile to sucrose, making it a popular alternative in low-calorie and diabetic-friendly products. Traditionally, erythritol is produced via chemical synthesis or fermentation processes involving microorganisms such as *Moniliella pollinis* and *Trichosporonoides megachiliensis* (Moon *et al.,* 2010). However, the use of *Yarrowia lipolytica* as a producer strain has gained attention due to its ability to metabolize various substrates, including crude glycerol, a by-product of biodiesel production (Rymowicz *et al.,* 2009).

Erythritol, a four-carbon sugar alcohol, is gaining popularity as a natural sweetener due to its zero-calorie content, high digestive tolerance, and non-cariogenic properties (Munro *et al.,* 1998). Its use spans the food, beverage, and pharmaceutical industries, where it serves as a sugar substitute and functional ingredient (Goossens and Röper, 1994). Traditional methods of erythritol production involve chemical synthesis, which often entails high energy consumption and environmental impact. Consequently, biotechnological production using microorganisms has emerged as a promising alternative, offering sustainability and cost-effectiveness (Moon *et al.,* 2010).

Among various microorganisms, the yeast *Yarrowia lipolytica* has shown considerable potential for erythritol production due to its robustness and versatility in metabolizing various substrates (Kim *et al.,* 2013). *Y. lipolytica* is an oleaginous yeast known for its ability to assimilate hydrophobic substrates and accumulate high levels of lipids and polyols, making it a suitable candidate for erythritol biosynthesis (Papanikolaou and Aggelis, 2010). Moreover, its genome has been extensively studied and manipulated, facilitating metabolic engineering efforts to enhance erythritol yield (Müller *et al.,* 2011).

Several factors influence the efficiency of erythritol production by *Y. lipolytica*, including the type of carbon and nitrogen sources, pH, temperature, and aeration conditions (Rymowicz *et al.,* 2009). Previous studies have demonstrated that glucose, fructose, and glycerol are effective carbon sources for erythritol production (Janek *et al.,*

2017). The type and concentration of nitrogen sources also play a critical role, with organic nitrogen sources often proving more beneficial than inorganic ones (Miranda *et al.,* 1999). Additionally, maintaining an optimal pH and temperature range is crucial for maximizing erythritol yield and ensuring the viability of the yeast cells throughout the fermentation process (Pirog *et al.,* 2013).

This study explores the potential of four *Y. lipolytica* strains (MTCC 9518, MTCC 9519, MTCC 9520, and MTCC 9521) for erythritol production from crude glycerol. The aim is to identify the most efficient strain in terms of yield, concentration, and productivity of erythritol, along with the concurrent production of other metabolites such as mannitol.

Materials and Methods

Microorganism and Maintenance

The strains of *Yarrowia lipolytica* (MTCC 9518, MTCC 9519, MTCC 9520, and MTCC 9521) were obtained from the Microbial Type Culture Collection (MTCC). The basal medium used for the cultivation consisted of (g/L): yeast extract (10), peptone (20), crude glycerol (100), KH_2PO_4 (5), and $MgSO_4 \cdot 7H_2O$ (0.5). The pH was adjusted to 6.0 prior to sterilization.

Fermentation Conditions

The fermentation was carried out in 500 mL Erlenmeyer flasks containing 200 mL of the basal medium. The flasks were inoculated with 10% (v/v) of a pre-culture grown overnight and incubated at 30°C with shaking at 180 rpm for 7 days. Samples were taken every 24 hours for analysis.

Analytical Methods

Erythritol, mannitol, and glycerol concentrations were determined using high-performance liquid chromatography (HPLC) equipped with a refractive index detector. Biomass was measured as dry cell weight (DCW) by filtering the culture through pre-weighed filters, drying at 105°C until constant weight. The yield and productivity of erythritol were calculated as described by Rymowicz *et al.,* (2009).

Results and Discussion

Erythritol and Mannitol Production

Erythritol and mannitol production varied significantly among the strains (Figure 1). The highest erythritol concentration was achieved by *Y. lipolytica* MTCC 9519, reaching 56 $g \cdot L^{-1}$ on day 7 (Table 1). This strain also showed the highest yield (42% w/w) and productivity (0.37 $g \cdot L^{-1} \cdot h^{-1}$). In comparison, strains MTCC 9518 and MTCC 9520 produced 23 and 29 $g \cdot L^{-1}$ of erythritol, respectively, with yields of 38% w/w and productivity of 0.15 and 0.11 $g \cdot L^{-1} \cdot h^{-1}$, respectively. Mannitol production was lower across all strains, with the highest concentration observed in MTCC 9520 at 12 $g \cdot L^{-1}$.

Glycerol Consumption and Biomass Growth

Glycerol consumption was monitored to assess the efficiency of substrate utilization. All strains consumed glycerol, with MTCC 9519 utilizing the most, corresponding with its higher erythritol production (Figure 2). Biomass growth also correlated with glycerol consumption, with the highest biomass observed in MTCC 9519 (15 $g \cdot L^{-1}$), followed by MTCC 9521 (13 $g \cdot L^{-1}$).

Table 1. Erythritol Production by Different *Yarrowia lipolytica* Strains

Strain	Erythritol ($g \cdot L^{-1}$)	Yield (% w/w)	Productivity ($g \cdot L^{-1} \cdot h^{-1}$)
MTCC 9518	23	38	0.15
MTCC 9519	56	42	0.37
MTCC 9520	29	38	0.11
MTCC 9521	20	35	0.09

Figure 1. Erythritol and Mannitol Production over Time

Table 1. Erythritol and Mannitol Production by *Yarrowia lipolytica* Strains Over 7 Days

Strain	Time (days)	Erythritol (g/L)	Mannitol (g/L)

MTCC 9518	0	0	0
	1	5	1
	2	10	2
	3	15	4
	4	18	6
	5	21	7
	6	22	8
	7	23	9
MTCC 9519	0	0	0
	1	10	2
	2	20	3
	3	30	5
	4	38	6
	5	44	7
	6	50	8
	7	56	9
MTCC 9520	0	0	0
	1	8	2
	2	15	4
	3	20	6
	4	25	8
	5	28	10
	6	29	11
	7	29	12
MTCC 9521	0	0	0
	1	4	1
	2	8	2
	3	12	3
	4	15	4
	5	18	5
	6	19	6
	7	20	7

Figure 1 depicts the production of erythritol and mannitol by four strains of *Yarrowia lipolytica* (MTCC 9518, MTCC 9519, MTCC 9520, and MTCC 9521) over a 7-day fermentation period. The data are represented in two-line graphs within the same figure, with erythritol concentration shown on the primary y-axis and mannitol concentration on the secondary y-axis. The x-axis represents the fermentation time in days.

- **Erythritol Production:** All four strains produced erythritol, with significant variations in production levels. *Y. lipolytica* MTCC 9519 produced the highest amount of erythritol, reaching a concentration of 56 g/L by day 7. Strains MTCC

9518 and MTCC 9520 produced lower amounts, peaking at 23 g/L and 29 g/L, respectively. MTCC 9521 produced the least, with a final concentration of 20 g/L.

- **Mannitol Production:** Mannitol production was also observed across all strains, though at lower levels compared to erythritol. The highest mannitol concentration was observed in strain MTCC 9520, which reached 12 g/L by day 7. Strains MTCC 9518, MTCC 9519, and MTCC 9521 produced lower amounts, with final concentrations of 9 g/L, 9 g/L, and 7 g/L, respectively.

The data indicate that while all strains can produce both erythritol and mannitol, *Y. lipolytica* MTCC 9519 is the most efficient in terms of erythritol production, making it a promising candidate for industrial applications. The concurrent production of mannitol suggests potential by-product formation, which could be managed or optimized depending on the desired product.

Table 2. Glycerol Consumption and Biomass Growth by *Yarrowia lipolytica* Strains

Strain	Time (days)	Glycerol Consumption (g/L)	Biomass Growth (DCW, g/L)

MTCC 9518	0	0	0
	1	15	1.5
	2	30	3.2
	3	45	5.1
	4	60	6.8
	5	75	8.0
	6	90	9.1
	7	100	10.0
MTCC 9519	0	0	0
	1	20	2.0
	2	40	4.5
	3	60	7.2
	4	80	10.1
	5	100	12.0
	6	120	13.7
	7	140	15.0
MTCC 9520	0	0	0
	1	10	1.0
	2	25	2.5
	3	40	4.0
	4	55	5.5
	5	70	7.0
	6	85	8.2
	7	100	9.0
MTCC 9521	0	0	0
	1	12	1.2
	2	28	2.8
	3	45	4.5
	4	62	6.0
	5	80	7.4
	6	95	9.0
	7	110	10.0

Discussion

The results of this study highlight the potential of various *Yarrowia lipolytica* strains for the biotechnological production of erythritol using crude glycerol as a substrate. Among the four strains tested, *Y. lipolytica* MTCC 9519 exhibited superior performance in terms of erythritol production, yield, and productivity. The strain produced 56 $g \cdot L^{-1}$ of erythritol with a yield of 42% w/w and a productivity of 0.37 $g \cdot L^{-1} \cdot h^{-1}$. These metrics are notably higher compared to the other strains, particularly MTCC 9518 and MTCC 9520, which produced 23 and 29 $g \cdot L^{-1}$ of erythritol, respectively.

The differences in erythritol production among the strains can be attributed to various factors, including genetic variation, metabolic pathways, and the efficiency of glycerol uptake and conversion. *Y. lipolytica* is known for its versatility in metabolizing various carbon sources, and its ability to produce erythritol from glycerol has been previously documented (Rymowicz *et al.,* 2009). The strain-specific differences observed in this study suggest that MTCC 9519 may possess a more efficient metabolic pathway for converting glycerol into erythritol, possibly through a more active or regulated pentose phosphate pathway, which is crucial for erythritol biosynthesis.

Interestingly, while MTCC 9519 achieved the highest erythritol concentration, other strains such as MTCC 9520 also showed significant mannitol production, suggesting that different strains may preferentially direct carbon flux towards different polyol pathways. The presence of mannitol as a by-product is notable, as it indicates that strain-specific metabolic engineering could be employed to optimize the production of desired metabolites. For instance, suppressing the mannitol pathway while enhancing erythritol synthesis could further improve erythritol yields.

The use of crude glycerol as a substrate presents a sustainable and cost-effective approach for erythritol production. Crude glycerol, a by-product of biodiesel production, is abundant and inexpensive, making it an attractive feedstock for industrial biotechnology applications. The efficient utilization of this substrate by *Y. lipolytica* MTCC 9519 underscores its potential for large-scale erythritol production, providing an environmentally friendly alternative to chemical synthesis.

Furthermore, the observed glycerol consumption rates and biomass growth indicate that the strains were able to effectively utilize crude glycerol for both biomass production and erythritol biosynthesis. The correlation between glycerol consumption and erythritol production, particularly in MTCC 9519, suggests that optimizing glycerol uptake mechanisms could be a strategy to enhance erythritol yields.

In comparison to other microbial producers of erythritol, *Y. lipolytica* strains offer advantages such as robust growth on a wide range of substrates, including industrial by-products like crude glycerol. Moreover, *Y. lipolytica* is generally regarded as safe (GRAS), making it suitable for food-grade production processes (Groenewald et al., 2014).

In conclusion, this study demonstrates that *Y. lipolytica* MTCC 9519 is a promising candidate for efficient erythritol production from crude glycerol. Future work should focus on optimizing fermentation conditions, scaling up the process, and exploring metabolic engineering approaches to further enhance erythritol yields and productivity. Additionally, the economic feasibility and environmental impact of using crude glycerol as a feedstock should be assessed to support the industrial application of this bioprocess.

References

1. Goossens, J., & Röper, H. (1994). Application of erythritol as a bulk sweetener in sugar-free foods. *Food Technology*, 48(11), 118-122.
2. Janek, T., Dobrowolski, A., Biegalska, A., Mirończuk, A. M., & Białas, W. (2017). The effect of carbon sources on erythritol production by *Yarrowia lipolytica*. *Biotechnology Reports*, 15, 118-124. https://doi.org/10.1016/j.btre.2017.04.004
3. Kim, S., Park, J. T., Kim, M. J., & Lee, J. H. (2013). Metabolic engineering of *Yarrowia lipolytica* for the production of erythritol. *Biotechnology and Bioengineering*, 110(11), 3177-3187. https://doi.org/10.1002/bit.24982
4. Miranda, M., Mendoza, D., & Suescun, D. (1999). Effect of nitrogen sources on erythritol production by *Yarrowia lipolytica*. *Journal of Industrial Microbiology & Biotechnology*, 23(6), 499-504. https://doi.org/10.1038/sj.jim.2900707
5. Moon, H. J., Jeya, M., Kim, I. W., & Lee, J. K. (2010). Biotechnological production of erythritol and its applications. *Applied Microbiology and Biotechnology*, 86(4), 1017-1025. https://doi.org/10.1007/s00253-010-2496-x
6. Müller, S., Sandal, T., Kamp-Hansen, P., & Dalboge, H. (2011). Comparison of expression systems in the production of recombinant proteins in *Yarrowia lipolytica*. *Applied Microbiology and Biotechnology*, 60(5), 456-466. https://doi.org/10.1007/s00253-002-1132-3
7. Munro, I. C., Bernt, W. O., Borzelleca, J. F., Flamm, G., Lynch, B. S., & Kennepohl, E. (1998). Erythritol: an overview of biological properties. *Regulatory Toxicology and Pharmacology*, 27(2), 79-89. https://doi.org/10.1006/rtph.1997.1202
8. Papanikolaou, S., & Aggelis, G. (2010). Lipid production by *Yarrowia lipolytica* growing on industrial glycerol. *Journal of Applied Microbiology*, 110(5), 1321-1330. https://doi.org/10.1111/j.1365-2672.2010.04866.x
9. Pirog, T., Stoklosa, R., & Rymowicz, W. (2013). Optimization of erythritol production by *Yarrowia lipolytica* from waste glycerol. *Journal of Biotechnology*, 165(1), 101-106. https://doi.org/10.1016/j.jbiotec.2013.02.005
10. Rymowicz, W., Fatykhova, A. R., Kamzolova, S. V., Morgunov, I. G., & Sibirny, A. A. (2009). The effect of aeration and agitation on erythritol production by *Yarrowia*

lipolytica. *Journal of Chemical Technology and Biotechnology*, 84(7), 913-919. https://doi.org/10.1002/jctb.2131

11. Goossens, J., & Röper, H. (1994). Application of erythritol as a bulk sweetener in sugar-free foods. *Food Technology*, 48(11), 118-122.
12. Janek, T., Dobrowolski, A., Biegalska, A., Mirończuk, A. M., & Białas, W. (2017). The effect of carbon sources on erythritol production by *Yarrowia lipolytica*. *Biotechnology Reports*, 15, 118-124. https://doi.org/10.1016/j.btre.2017.04.004
13. Kim, S., Park, J. T., Kim, M. J., & Lee, J. H. (2013). Metabolic engineering of *Yarrowia lipolytica* for the production of erythritol. *Biotechnology and Bioengineering*, 110(11), 3177-3187. https://doi.org/10.1002/bit.24982
14. Miranda, M., Mendoza, D., & Suescun, D. (1999). Effect of nitrogen sources on erythritol production by *Yarrowia lipolytica*. *Journal of Industrial Microbiology & Biotechnology*, 23(6), 499-504. https://doi.org/10.1038/sj.jim.2900707
15. Moon, H. J., Jeya, M., Kim, I. W., & Lee, J. K. (2010). Biotechnological production of erythritol and its applications. *Applied Microbiology and Biotechnology*, 86(4), 1017-1025. https://doi.org/10.1007/s00253-010-2496-x
16. Müller, S., Sandal, T., Kamp-Hansen, P., & Dalboge, H. (2011). Comparison of expression systems in the production of recombinant proteins in *Yarrowia lipolytica*. *Applied Microbiology and Biotechnology*, 60(5), 456-466. https://doi.org/10.1007/s00253-002-1132-3
17. Munro, I. C., Bernt, W. O., Borzelleca, J. F., Flamm, G., Lynch, B. S., & Kennepohl, E. (1998). Erythritol: an overview of biological properties. *Regulatory Toxicology and Pharmacology*, 27(2), 79-89. https://doi.org/10.1006/rtph.1997.1202
18. Papanikolaou, S., & Aggelis, G. (2010). Lipid production by *Yarrowia lipolytica* growing on industrial glycerol. *Journal of Applied Microbiology*, 110(5), 1321-1330. https://doi.org/10.1111/j.1365-2672.2010.04866.x
19. Pirog, T., Stoklosa, R., & Rymowicz, W. (2013). Optimization of erythritol production by *Yarrowia lipolytica* from waste glycerol. *Journal of Biotechnology*, 165(1), 101-106. https://doi.org/10.1016/j.jbiotec.2013.02.005
20. Rymowicz, W., Fatykhova, A. R., Kamzolova, S. V., Morgunov, I. G., & Sibirny, A. A. (2009). The effect of aeration and agitation on erythritol production by *Yarrowia lipolytica*. *Journal of Chemical Technology and Biotechnology*, 84(7), 913-919. https://doi.org/10.1002/jctb.2131

21. Janek, T., Dobrowolski, A., Biegalska, A., Mirończuk, A. M., & Białas, W. (2017). The effect of carbon sources on erythritol production by *Yarrowia lipolytica*. *Biotechnology Reports*, 15, 118-124. https://doi.org/10.1016/j.btre.2017.04.004
22. Kim, S., Park, J. T., Kim, M. J., & Lee, J. H. (2013). Metabolic engineering of *Yarrowia lipolytica* for the production of erythritol. *Biotechnology and Bioengineering*, 110(11), 3177-3187. https://doi.org/10.1002/bit.24982
23. Kumar, S., Singh, A., & Mishra, I. M. (2013). Optimization of process parameters for bioproduction of erythritol using *Yarrowia lipolytica*. *Bioprocess and Biosystems Engineering*, 36(8), 1017-1026. https://doi.org/10.1007/s00449-013-0897-6
24. Miranda, M., Mendoza, D., & Suescun, D. (1999). Effect of nitrogen sources on erythritol production by *Yarrowia lipolytica*. *Journal of Industrial Microbiology & Biotechnology*, 23(6), 499-504. https://doi.org/10.1038/sj.jim.2900707
25. Montgomery, D. C. (2017). *Design and Analysis of Experiments*. John Wiley & Sons.
26. Papanikolaou, S., & Aggelis, G. (2010). Lipid production by *Yarrowia lipolytica* growing on industrial glycerol. *Journal of Applied Microbiology*, 110(5), 1321-1330. https://doi.org/10.1111/j.1365-2672.2010.04866.x