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REVIEW ARTICLE **OPEN ACCESS**

Strain Improvement for Citric Acid Production from Raw Glycerol using *Yarrowia lipolytica*

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ABSTRACT

Glycerol is in abundant supply on the market as a result 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. Citric Acid was initially extracted from citrus, but due to the low efficiency of the procedure and the rising demand, researchers soon began looking for more effective ways to make Citric Acid. Currently, fermentations involving microbes, particularly filamentous fungi and yeasts, provide 99% of the world's Citric Acid requirement. Yarrowia lipolytica yeast strains have the capacity to develop on culture media using glycerol from the biodiesel sector and produce citric acid, which has numerous industrial uses. Mutagenesis & selection can be used to improve yeast strains that produce citric acid. Alternative production methods and strains are being used to meet the annual increases in demand for citric acid.

Keywords: *Glycerol, biodiesel, Citric Acid, Yarrowia lipolytica, Fermentation, strain improvement, yeast strains*

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INTRODUCTION

Bio fuels are fuel alternatives to fossil fuels that, in addition to being sustainable & biodegradable, also offer the benefit of lowering oil imports and lowering the emissions of CO (Carbon monoxide), hydrocarbons, soot, as well as particulates [1]. Biodiesel is one type of bio fuels that is frequently manufactured, and as a by-product of its production, other chemicals are also synthesized. The quantity of Glycerol is the principal by-product of this process of production, accounting for 10% of the biodiesel produced, and the amount of these extra products generated is expanding as a result of increased output [2]. The quest for substitutes for this by-product has been prompted by the significant amount of glycerol that is produced during the manufacturing of bio fuels [3]. The utilization of glycerol as a source of carbon and energy for the manufacturing of chemicals of commercial importance has been highly alluring in order to reduce environmental concerns with the build-up of glycerol & lower the cost of biodiesel synthesis. Citric acid is one of the organic acids that can be generated using the microbial method and glycerol as the source of carbon [4,5].

Production of Citric Acid by Yeasts

In the food, pharmaceutical, and beverage sectors, citric acid is indeed a commonly utilized organic acid with high commercial value. The food sector uses it as the primary additive. To give dishes and beverages a pleasant, tart flavour, citric acid is frequently utilized. Additionally, it serves as an emulsifier, preservative, antioxidant, acidulant, or antioxidant in the formulation of numerous foods. About 70% of citric acid's usage is in the food business, with the remaining 10% going to the pharmaceutical and cosmetic industries [6]. Because citric acid is less hazardous than other acidulants, there is a high demand for it on a global scale. According to reports, there is a very limited availability of natural citric acid, and biotechnology fermentation techniques are the only way to meet the demand. According to reports, fermentation is used to manufacture and over 90% of the citric acid that is produced globally [7]. Three methods can be employed to produce citric acid in mass quantities: submerged fermentation, surface fermentation, and solid/state fermentation, or the "Koji" technique [8]. According to estimates, submerged fermentation using stirred tanks with a size of 40/200 m^3 or more, or large airlift fermentors with a capacity of 200/900 m^3, is around 80% of the world's citric acid is manufactured [9]. While continuous systems, fed-batch systems, and batch systems can all be utilized for submerged fermentation,

the batch mode is the most common. The only method for producing citric acid by yeasts is submerged cultivation. The submerged fermentation process is preferred because it is more effective and more amenable to automatization [10]. In addition to the traditional batch method, continuous culture with immobilized cells has also been utilized to produce citric acid from a variety of strains, the majority of which are members of the genus *Candida (Yarrowia)* [11]. *Yarrowia lipolytica, Candida oleophila, C. paratropicalis, C. catenulata, C. guilliermondii, C. intermedia, C. zeylanoides, Pichia anomala, C. parapsilosis*, & several *Rhodotorula* species are the yeast species that are known to produce citric acid [12]. *Yarrowia lipolytica*, a species of yeast, has recently been discovered as a microbial cell factory to produce citric acid and is recognized as a possible producer of the acid [13]. The following are some of the primary benefits of employing yeasts: Compared to fungi, yeasts are more resistant to high substrate concentrations, have similar conversion rates, and have a higher tolerance for metal ions, allowing them to utilize less refined substrates. Due to yeasts' unicellular nature, they also provide for improved process control [14]. According to a report, yeast could replace *Aspergillus niger* as a source of citric acid in the future, particularly if a yeast biomass is added to animal food rather than used as a by-product. The first stage in producing citric acid has been reported to be choosing a yeast strain that produces large amounts of citric acid and has high citric acid: isocitric acid ratios [15].

Citric Acid Synthesis in *Yarrowia lipolytica*

Non-conventional yeast, Yarrowia lipolytica is phylogenetically distinct from *Saccharomyces cerevisiae* and other well-researched yeast species. It is a member of the *Hemiascomycetes* family [16]. The American Food and Drug Administration have classified *Yarrowia lipolytica* as GRAS, a non-pathogenic bacterium (FDA). Since no reproductive state had been documented until the middle of the 1960s, when the ideal form was found to have two mating kinds, Yarrowia lipolytica was once thought to belong to Candida genus (A and B) [17]. The ability of the species to hydrolyze lipids is where the term "lipolytica" comes from. Y. lipolytica exhibits dimorphism in terms of morphology, which indicates that this fungus can produce yeast cells, pseudohyphae, & septate hyphae [18]. The major cell types vary depending on the strain and certain environmental factors. In this regard, some carbon sources, such as oleic acid, oleic alcohol, or linoleic acid, along with some nitrogen sources, like meat extract, might encourage the development of mycelium [19]. Diverse colony morphologies, ranging from smooth & shiny to severely convoluted and mat can also be caused by strains and growing circumstances. A great model for producing numerous biotechnological products is *Yarrowia lipolytica*. For instance, Y. lipolytica creates pyruvic acid, TCA intermediates (CA, α-ketoglutarate, ICA, & succinic acid), and amino acids like lysine, proteins & enzymes including RNases, esterases, phosphatases, lipases, and alkaline and acid proteases, all of which have high economic value [20].

As a by-product of the TCA cycle, Citric Acid is synthesized in viable cells. However, some fungi and bacteria can accumulate it by a cyclic abnormality. The procedure is known as Fermentation of the Gaden type II, in which CA is synthesized through primary metabolism but is not associated with growth [21]. Since CA formation begins when accessible nitrogen has been utilized, a lack of nitrogen sources is the most crucial prerequisite for CA deposition in yeast. The amount of ammonium ions in the medium has a negative impact on citrate synthase, the enzyme that produces CA from oxaloacetate and acetyl-CoA [22].

The nitrogen source is exhausted however the carbon source is still in excess in carbon-excess (nitrogen-limited) conditions. When glycerol, or another form of digested extra carbon, is present. When nitrogen is scarce, the rate of catalytic growth declines quickly whereas the rate of carbon assimilation declines gradually. Due to this, the release of Citric Acid (or other low-molecular-weight molecules) into the growth medium or storage lipid neo-synthesis, which results in so-called de novo lipid accumulation, is the preferred channelling of the carbon flux. In these circumstances, *Y. lipolytica* synthesizes large amounts of TCA cycle intermediates such Citric Acid and Iso-Citric Acid [23].

Type of strain utilised is one of the key parameters for Citric acid generation. Different collections of Y. lipolytica contain a variety of wild strains, including W, ATCC, NRRL, NCIM, VKM, LGAM, UFLA, NCYC, ACA-YC, and NBRC 1. Different organisations were able to create inbred strains from the French, German, and American strains [17]. Citric acid production is significantly impacted by concentration and the kind of source of carbon used in Citric Acid production. It is known that wild, recombinant & mutant strains of *Y. lipolytica* may utilize a wide range of carbon sources, including pure or raw glycerol, animal fats, molasses, alcohols (ethanol and methanol), starch hydrolysates, fructose, and glucose [24]. The synthesis of Citric Acid has used raw glycerol, which is a by-product of the biodiesel industry, as a carbon source. According to estimates, the production of 10 kg biodiesel results in the production of 1 kg of raw glycerol [25]. Numerous research studies have employed it to produce Citric Acid due to its inexpensive cost and high Citric Acid conversion yield as the best carbon source. In batch bioreactor culture, Rymowicz et al. (2006) used an acetate mutant of *Y. lipolytica* to achieve the highest Citric Acid titre of 124.5 g/L from

an early raw glycerol concentration at 200 g/L. Later, fed-batch growth was used, and from a starting glycerol concentration of 300 g/L, a maximum Citric Acid output of 157.5 g/L was attained [26]. Furthermore, if both carbon substrates were available in the medium, several experiments found that *Y. lipolytica* produced Citric Acid more effectively from glycerol than from glucose. This was explained by the fact that glycerol (C3), which could not inhibit C6 transporters because of their distinct carriers, reduced the activity of the C6 pathway during glycolysis [27].

Strain Improvement for Improved Production of Citric Acid

Citric acid productivity can be increased up to a certain level by applying traditional methods of fermentation using stirred vessels and adjusting fermentation conditions. It is well known that yeasts' ability to produce citric acid depends heavily on strain diversity [28]. Several strain/screening experiments have shown this phenomenon to exist. Basically, the process of choosing strains involves isolating them from their natural environments using conventional microbiological techniques, and then testing them for their capacity to produce citric acid [29]. A number of these strains have been added to official or commercial culture collections. In addition, discovering new yeast strains that could be employed in methods to produce citric acid has become a recent interest among researchers. Biological or physical parameters must be altered in order to increase the productivity of the process and the yield of citric acid. In this regard, strain improvement has taken on importance [30]. By using mutagenesis & selection, citric acid-producing microorganisms have been improved. The method that has been used the most frequently is to use mutagens to cause mutations in parental strains. Chemical mutagens, UV irradiation, and /irradiation are the most often employed mutagens. According to reports, UV therapy and various chemical mutagens are regularly coupled. Before strains with increased performance can be isolated, chemical and physical mutagen treatments must be applied repeatedly, accompanied by screening a significant number of colonies [31].

Several crucial criteria must be considered when choosing mutants or strains for large-scale production. These include the ability of strains to remain stable during subculture for mass propagation without experiencing physiological or biochemical degeneration, the inability to utilize the acid produced, and the absence of the production of additional metabolic acids like malic, gluconic, and oxalic acid. In a previously published work, *Y. lipotytica* Y-1095 was exposed to UV/irradiation and (NTG) N-methyl-NT-nitro-N-nitrosoguanidine, which are two distinct mutagens [32]. It was shown that UV/irradiation was more effective at producing more effective isolates. Researchers chose four mutants, each of which produced approximately 75–80% more citric acid than the original parent. A UV/induced mutant of Yarrowia lipolytica was discovered to be the most suited for citric acid generation from glucose hydrol in a study including four commercial strains & two mutants [28].

The majority of Yarrowia lipolytica strains may grow effectively when given acetate as their only carbon source. Several mutants that were prevented from using acetate have been identified and documented. According to a report, the use of acetate is connected to the activation of glyoxylate pathway, which is crucial for the metabolism of citric acid. Mutants that had their acetyl/coenzyme A synthetase activity stopped were studied. The glyoxylate cycle cannot be induced in acetyl-coenzyme A deficient mutants because acetyl/coenzyme A is required for its induction [33]. (Qian et al., 2020). Rymowicz et al. employed three Yarrowia lipolytica acetate/negative mutants to produce citric acid from raw glycerol, and strain 1.31 produced the highest quantity of citric acid—124.5 g/L [34].

CONCLUSION

Yeast strains are employed in industrial operations nowadays and are capable of producing citric acid. According to reports, citric acid consumption is rising by 5% annually. Alternative cultivation techniques using Yarrowia strains are employed to produce citric acid due to the high and continuing demand for it. The prospect of obtaining various Yarrowia strains that yield good yields of citric acid is of great interest, in addition to process optimization. Mutation and selection research can be used to improve citric acid-producing Yarrowia strains. After exposing the genomic material to chemical or physical mutagenic agents, Yarrowia strains with superior traits can be chosen, such as increased citric acid output and fermentation rate. Further research should be focused to choosing a higher citric acid producing Yarrowia strain and improving its metabolic pathways and operating settings in order to make citric acid generation by yeasts more commercially viable.

REFERENCES:

1. Mwangi, J. K., Lee, W. J., Chang, Y. C., Chen, C. Y., & Wang, L. C. (2015). An overview: Energy saving and pollution reduction by using green fuel blends in diesel engines. Applied Energy, 159: 214-236.

2. Tan, H. W., Aziz, A. A., & Aroua, M. K. (2013). Glycerol production and its applications as a raw material: A review. Renewable and sustainable energy reviews, 27: 118-127.
3. Rahman, M. S., Xu, C. C., Ma, K., Guo, H., & Qin, W. (2017). Utilization of by-product glycerol from bio-diesel plants as feedstock for 2, 3-butanediol accumulation and biosynthesis genes response of Klebsiella variicola SW3. Renewable Energy, 114:1272-1280.
4. Ahmad, I., Abdullah, N., Koji, I., Yuzir, A., Mohamad, S. E., Show, P. L., & Khoo, K. S. (2022). The role of restaurant wastewater for producing bioenergy towards a circular bioeconomy: A review on compositions, environmental impacts, and sustainable integrated management. Environmental Research, 113854.
5. Kumar, L. R., Yellapu, S. K., Yan, S., Tyagi, R. D., & Drogui, P. (2021). Elucidating the effect of impurities present in different crude glycerol sources on lipid and citric acid production by Yarrowia lipolytica SKY7. Journal of Chemical Technology & Biotechnology, 96(1): 227-240.
6. Ciriminna, R., Meneguzzo, F., Delisi, R., & Pagliaro, M. (2017). Citric acid: emerging applications of key biotechnology industrial product. Chemistry Central Journal, 11(1): 1-9.
7. Show, P. L., Oladele, K. O., Siew, Q. Y., Aziz Zakry, F. A., Lan, J. C. W., & Ling, T. C. (2015). Overview of citric acid production from *Aspergillus niger*. Frontiers in life science, 8(3):271-283.
8. Lende, S. V., Karemore, H., & Umekar, M. J. (2021). Review on production of citric acid by fermentation technology. *GSC* Biological and Pharmaceutical Sciences, 17(3):085-093.
9. Börekçi, B. S., Kaban, G., & Kaya, M. (2021). Citric acid production of yeasts: an overview. The EuroBiotech Journal, 5(2):79-91.
10. Arora, S., Rani, R., & Ghosh, S. (2018). Bioreactors in solid state fermentation technology: Design, applications, and engineering aspects. Journal of Biotechnology, *269*:16-34.
11. Hesham, A. E. L., Mostafa, Y. S., & AlSharqi, L. E. O. (2020). Optimization of citric acid production by immobilized cells of novel yeast isolates. Mycobiology, 48(2): 122-132.
12. da Veiga Moreira, J. (2019). Modélisation de la bascule métabolique chez les cellules eucaryotes: application à la production de citrate chez la levure Yarrowia lipolytica (Doctoral dissertation, Université Paris Saclay (COmUE)).
13. Sabra, W., Bommareddy, R. R., Maheshwari, G., Papanikolaou, S., & Zeng, A. P. (2017). Substrates and oxygen dependent citric acid production by *Yarrowia lipolytica*: insights through transcriptome and fluxome analyses. Microbial cell factories, 16(1):1-14.
14. Kumar, D., Singh, B., & Korstad, J. (2017). Utilization of lignocellulosic biomass by oleaginous yeast and bacteria for production of biodiesel and renewable diesel. Renewable and Sustainable Energy Reviews, 73:654-671.
15. Ferreira, J. A., Mahboubi, A., Lennartsson, P. R., & Taherzadeh, M. J. (2016). Waste biorefineries using filamentous ascomycetes fungi: present status and future prospects. Bioresource Technology, 215:334-345.
16. Nicaud J. M. (2012). *Yarrowia lipolytica*. Yeast (Chichester, England), 29(10):409–418. https://doi.org/10.1002/yea.2921
17. Cavallo, E., Charreau, H., Cerrutti, P., & Foresti, M. L. (2017). Yarrowia lipolytica: a model yeast for citric acid production. FEMS yeast research, 17(8): 10.1093/femsyr/fox084. https://doi.org/10.1093/femsyr/fox084
18. Walker, G. M., & White, N. A. (2017). Introduction to fungal physiology. Fungi: biology and applications, 1-35
19. Dobrowolski, A., Mituła, P., Rymowicz, W., & Mirończuk, A. M. (2016). Efficient conversion of crude glycerol from various industrial wastes into single cell oil by yeast Yarrowia lipolytica. Bioresource technology, 207:237-243.
20. Kövesi, Z. (2020). Biotechnological production of value-added chemicals from cis-aconitate with the help of genetically engineered oleophilic yeasts.
21. Carsanba, E., Papanikolaou, S., Fickers, P., Agirman, B., & Erten, H. (2019). Citric acid production by Yarrowia lipolytica. In Non-conventional yeasts: from basic research to application (pp. 91-117). Springer, Cham.
22. Morgunov, I. G., Kamzolova, S. V., & Lunina, J. N. (2013). The citric acid production from raw glycerol by Yarrowia lipolytica yeast and its regulation. Applied microbiology and Biotechnology, 97(16):7387-7397.
23. 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.
24. Çelik, E., & Çalık, P. (2012). Production of recombinant proteins by yeast cells. Biotechnology advances, 30(5):1108-1118.
25. Leoneti, A. B., Aragão-Leoneti, V., & De Oliveira, S. V. W. B. (2012). Glycerol as a by-product of biodiesel production in Brazil: alternatives for the use of unrefined glycerol. Renewable Energy, 45:138-145.
26. Rymowicz, W., Rywińska, A., Żarowska, B., & Juszczyk, P. (2006). Citric acid production from raw glycerol by acetate mutants of *Yarrowia lipolytica*. Chemical Papers, 60(5):391-394
27. Guo, Z., Duquesne, S., Bozonnet, S., Cioci, G., Nicaud, J. M., Marty, A., & O'Donohue, M. J. (2015). Development of cellobiose-degrading ability in *Yarrowia lipolytic*a strain by overexpression of endogenous genes. Biotechnology for biofuels, 8(1):1-16.
28. Madzak, C. (2021). *Yarrowia lipolytica* strains and their biotechnological applications: how natural biodiversity and metabolic engineering could contribute to cell factories improvement. Journal of Fungi, 7(7):548.
29. Vandenberghe, L. P. S., Rodrigues, C., De Carvalho, J. C., Medeiros, A. B. P., & Soccol, C. R. (2017). Production and application of citric acid. In Current developments in biotechnology and bioengineering (pp. 557-575). Elsevier.
30. Behera, B. C., Mishra, R., & Mohapatra, S. (2021). Microbial citric acid: Production, properties, application, and future perspectives. Food Frontiers, *2*(1):62-76.
31. Steensels, J., Snoek, T., Meersman, E., Nicolino, M. P., Voordeckers, K., & Verstrepen, K. J. (2014). Improving industrial yeast strains: exploiting natural and artificial diversity. FEMS microbiology reviews, *38*(5):947-995.

32. Morgunov IG, Kamzolova SV, Karpukhina OV, Bokieva SB, Lunina JN, Inozemtsev AN. (2020). Microbiological Production of Isocitric Acid from Biodiesel Waste and Its Effect on Spatial Memory. Microorganisms. 25;8(4):462. doi: 10.3390/microorganisms8040462. PMID: 32218311; PMCID: PMC7232500.
33. Qian, X., Xu, N., Jing, Y., Song, M., Zhou, J., Dong, W., & Ochsenreither, K. (2020). Valorization of crude glycerol into citric acid and malic acid by *Yarrowia lipolytica*. Industrial & Engineering Chemistry Research, 59(39):17165-17172.
34. Rymowicz, W., Rywińska, A., & Marcinkiewicz, M. (2009). High-yield production of erythritol from raw glycerol in fed-batch cultures of *Yarrowia lipolytica*. Biotechnology Letters, *31*(3):377-380.

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