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MODULATED LACTIC ACID PRODUCTION IN SACCHAROMYCES CEREVISIAE BY OXYGEN SUPPLY

Laura Camesasca[1], Silvia Batista[2] & Claudia Lareo[1]*

[1] *Dpto de Bioingeniería, Instituto de Ingeniería Química, Facultad de Ingeniería, UdelaR, Montevideo, Uruguay.*
[2] *Departamento de Bioquímica y Genómica Microbiana, Microbiología Molecular, IIBCE, Montevideo, Uruguay.*
** Corresponding author's email address: clareo@fing.edu.uy*

ABSTRACT

Lactic acid, is one of the most widely produced organic acids. It is used for different applications, being one of the most attractive one, its use in the synthesis of the biopolymer PLA. Even if it can be produced by many bacteria, the use of yeast results an interest option, for their resistance to acid environment and low growing requirements. Besides their advantages, homolactic fermentation presents difficulties in anaerobic conditions. This work evaluates the influence of different conditions of oxygen supply in lactic acid production in a modified yeast BY4741 lucking the genes PDC1 and ADH1 and expressing a bovine lactate dehydrogenase in a high copy number plasmid. The results showed that oxygen can modulate lactic acid production.

Keywords: Lactic acid. *Saccharomyces cerevisiae*. Oxygen.

1 INTRODUCTION

Lactic acid is an organic acid widely used on an industrial scale. It has various applications such as acidulant, food preservative, flavor enhancer in the food industry, as well as a pH regulator and green solvent in the chemical industry. Additionally, it is used as moisturizer, skin lightening agent, and humectant in the cosmetic industry, among other uses[1]. In recent years, lactic acid production has garnered increased interest due to its role as a monomer for poly lactic acid (PLA). PLA is highly valued for its elasticity, rigidity, thermoplastic behavior, and biocompatibility, making it one of the most promising biopolymers to replace petroleum-based polymers[2].

Lactic acid most known producers are the lactic acid bacteria (LAB). However, there are many other microorganisms such as other bacteria, yeast, filamentous fungi, microalgae and cyanobacteria, that can also produce lactic acid[1]. Yeasts, although their wild-type production is generally low in most cases, can be genetically engineered to achieve higher productivity. Since yeasts can tolerate high acidic environments compared to LAB, which are inhibited by high acidic levels, yeasts can potentially reduce production costs by eliminating the need for neutralizers [3, 4].

Saccharomyces cerevisiae has been widely used in the genetic engineering for lactic acid production because it has many features such as its robustness and fermentative capacity, as well as the knowledge of its genome and biochemical routes, that make it an attractive model[4]. In most of the genetic editions the genes involved in ethanol production (*PDC1*, *PDC5*, *PDC6*, *ADH1*, *ADH3*, *ADH4* and *ADH5*) or combination of them are deleted. This can lead in some cases to growth difficulties or energetic imbalance [5, 6]. This is why, besides the genetic modifications, employing culture strategies that promote lactic acid production while reducing the formation of other by-products such as glycerol, acetic acid, and/or ethanol is beneficial. Some works have proved that oxygen can enhance homolactic acid fermentation in *Saccharomyces*. This may be attributed to ATP requirement for transporting lactic acid out of the cell[7, 8]. In this work, it is evaluated how oxygen affects lactic acid production in the *Saccharomyces cerevisiae* strain BY4741Δ*PDC*1Δ*ADH*1, which expresses a lactate dehydrogenase from Boss Taurus in a high copy number plasmid.

2 MATERIAL & METHODS

Strain used: Haploid strain (Mat a) of *Saccharomyces cerevisiae* BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0), expressing a bovine lactate dehydrogenase in a high copy number plasmid. The *PDC1* gene has been deleted by the insertion of the *MET15* gene and *ADH1* gene has been deleted by the insertion of the KANMX cassette that confers resistance to the G418 antibiotic.

Culture media contained: glucose 65 g/L, $(NH_4)_2SO_4$ 5 g/L, leucine 50 mg/L, histidine 20 mg/L and yeast nitrogen base 1.7 g/L.

Fermentation conditions: The aeration was regulated by adjusting the filling level of the flasks and the supply of gaseous nitrogen. Four aeration conditions were evaluated: filling ratio of 0.25 with cotton plug (ST), filling ratio of 0.25 with cotton plug for 24 hours followed by venting with N_2 and then hermetically sealed (CT24), filling ratio of 0.25 with cotton plug for 48 hours followed by venting with N_2 and then hermetically sealed (CT48), and filling ratio of 0.05 with cotton plug (AE). Agitation was kept at 200 rpm in an orbital shaker.

3 RESULTS & DISCUSSION

Engineered *Saccharomyces cerevisiae*, as it has been studied by many groups, has difficulties producing lactic acid in a homolactic fermentation in anaerobic conditions [6, 7]. In this work, different conditions of oxygen supply were studied in order to evaluate the oxygen levels needed as well as the period of time of oxygen supply that favors the lactic acid production. In Figure 1, the results are presented for the sets of fermentation conditions stated in materials and methods

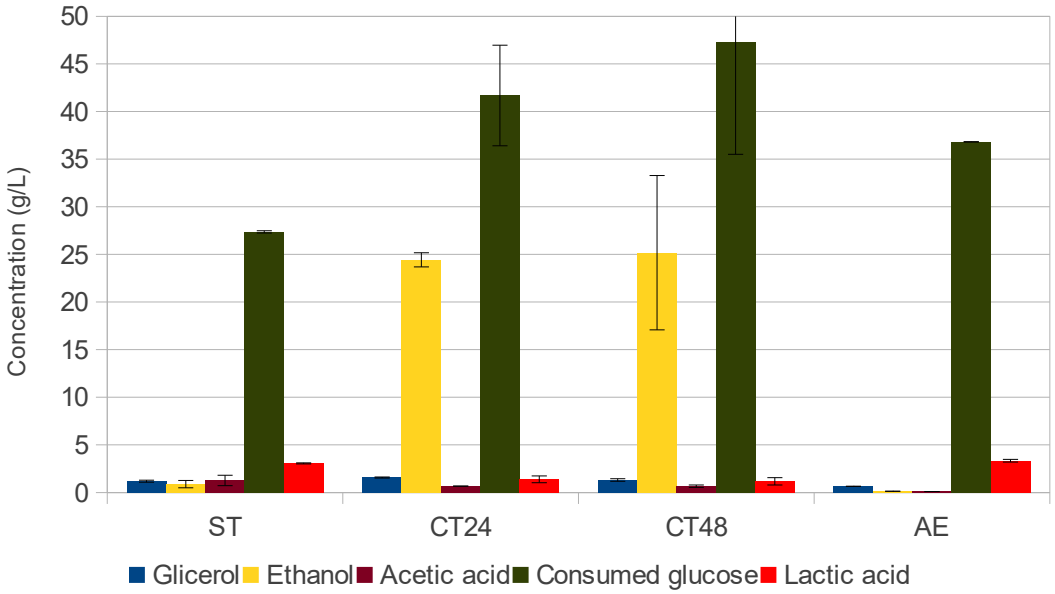

Figure 1 Lactic acid production under different oxygen supply conditions.

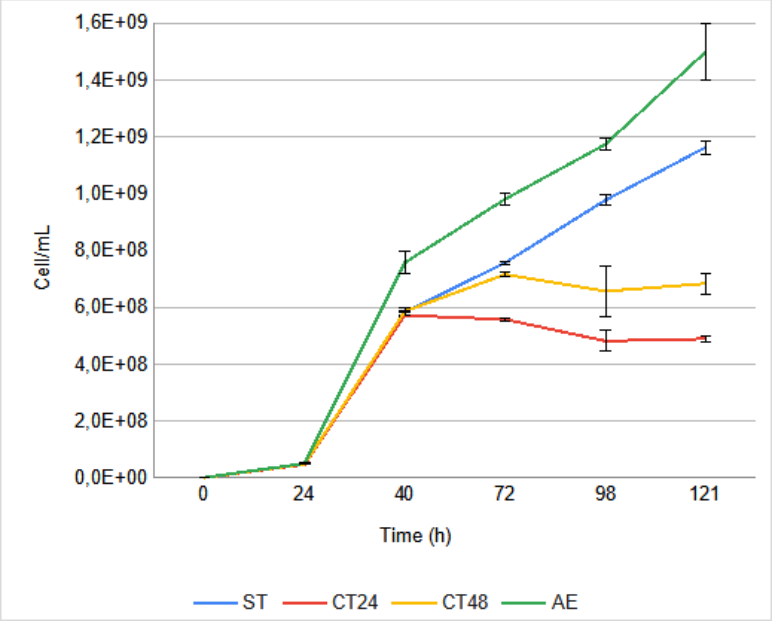

Figure 2 Growth curve for the studied conditions.

As it can be observed in Figure 1, the most aerated conditions favored the lactic acid production compared to the conditions with anaerobic stages. The conditions CT24 and CT48, with a filling relation of 0,25 with an oxygenated stage of 24 and 48 hours respectively, showed no significant differences in their lactic acid and ethanol profile. CT48 showed a higher glucose consumption compared to CT24, which could derive into higher cell growth. The most aerated conditions AE and ST produced 2,8 and 2,6 times more lactic acid than the other conditions. Between these two conditions, there are no significant differences in the lactic acid production. However, the most aerated condition AE has almost negligible production of the other fermentation products (acetic acid, ethanol and glycerol). So, even if a more oxygenated condition may not result in higher lactic production, it reduces subproduct generation. This behavior of lactic acid production in engineered *S. cerevisiae*, was explained by other

authors suggesting the generated ATP in the lactic acid fermentation is consumed to export the lactic acid out of the cell, having no net ATP gaining [8,9]. Those, culture conditions with microaerobic conditions could favor lactic acid production.

CONCLUSION

The production of lactic acid by yeast is attractive due to their robustness and low nutrient requirements. However, to achieve the production of lactic acid, its genetic modification is necessary, as well as the use of strategies that favor lactic acid fermentation. In this work, we used a strain that was modified by deleting the *PDC1* and *ADH1* genes and express a lactate dehydrogenase in a high copy number plasmid. As reported in the literature, *Saccharonmyces* has difficulties in achieving homolactic fermentation, due to the use of ATP for the transport of lactic acid. In this work the modulation in the production of lactic acid and the generation of byproducts was evaluated (ethanol, acetic acid and glycerol) through variations in oxygen supply. From these results it is concluded that the production of lactic acid is favored in conditions of greater oxygenation, also reducing the generation of byproducts. More studies are needed to adjust the exact oxygen levels that maximize lactic acid production.

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