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Growth Kinetics of *Yarrowia lipolytica* Engineered for Geraniol Production

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ABSTRACT

Geraniol, commonly extracted from plant essential oils, is a promising natural compound that demonstrates significant pharmacological properties. The yeast Yarrowia lipolytica, has a remarkable capacity for intracellular lipid accumulation and resistance to organic compounds, which is appropriate for biosynthesis of terpenes like geraniol. This study presents a kinetic comparison of three Y. lipolytica strains: W29 (wild type), ST9202 (W29 engineered for monoterpene precursor production), and BYa3105 (ST9202 engineered with geraniol synthase gene from Catharanthus roseus for geraniol biosynthesis). The wild strain W29 exhibited the highest specific growth rate (μmax = 0,593 h^{-1}) and biomass concentration (9,75 g/L). Strains ST9202 and BYa3105 showed reduced growth rates, with μmax values of 0,517 h^{-1} and 0,509 h^{-1}, respectively, along with lower biomass accumulation as compared to the parental strain. Specific substrate consumption rates (μS) were similar for W29 (0,445 h^{-1}) and ST9202 (0,415 h^{-1}), while BYa3105 had the highest (μS = 0,500 h^{-1}) despite its lowest substrate-to-cell conversion rate (Yx/s = 1,02 gX gS-1). These findings indicate that metabolic diversion towards terpene production in the modified strains affects their growth kinetics, but addition of the geraniol synthase gene does not compromise further the microbial physiological parameters.

Keywords: Geraniol. Metabolic Engineering. Terpene Production. Genetic modification.

1 INTRODUCTION

Geraniol is a promising natural substance that has been known to exhibit pharmacological properties such as antitumor activities and anti-microbial, that can be applied in the medical field. The monoterpene is commonly obtained by extracting essential oils from plants and isolating the molecule by fractional distillation. Since plant growth is highly dependent on climatic conditions, geraniol production through extraction is expensive and has little reproducibility to meet the huge global demand.[1,2] Some studies on genetically modified microorganisms such as bacteria and yeast have shed light on the potential as an alternative to terpene production.[3]

In terms of enhancing industrial production with microorganisms, the design of yeast strains and the integration of omic analyses provides valuable insights for the improvement of metabolic models and development of new strains, thereby optimizing overall productivity. Metabolic engineering method employs metabolic modeling to streamline fermentation process whilst taking into consideration growth parameters. [3,4] Yeast is a valuable production system due to its significant advantages, such as, rapid growth, simple genetic manipulation, and efficient fermentation capabilities. Systems biology in this approach is essential for effectively exploiting genetic modification across different types of yeast.[4,5]

The yeast *Yarrowia lipolytica* is a microorganism characterized by distinguished traits that make it a highly promising candidate for the production of industrially significant compounds, such as terpenes. Renowned for its ability to accumulate intracellular lipids, *Y. lipolytica* exhibits a remarkable resistance to organic compounds and elevated levels of cytosolic acetyl-CoA, making it particularly well-suited for the sustainable and efficient synthesis of terpenes, such as geraniol. [6,7]

In order to qualify as a viable alternative and further harness this quality, it is necessary to assess the effects of genetic modifications of *Y. lipolytica* aimed at enhancing terpene production on both growth kinetics and substrate consumption. To this end, three strains were selected and cultivated in a batch setup, W29, a wild strain, ST9202 modified to produce monoterpene precursors and BYa3105, a strain derived from the ST9202 strain engineered for heterologous expression of the geraniol synthase gene (*GES*) from *Catharanthus roseus*.[7]

2 MATERIAL & METHODS

For inoculum preparation, the three strains of *Y. lipolytica* (W29, ST9202 and BYa3105) from frozen stock cultures plated on solid medium YPD were cultivated in 10 g/L of yeast extract, 20 g/L of bacteriological peptone and 20 g/L of glucose, sterilized in an autoclave at 121ºC for 20 minutes and incubated overnight in an incubator at 30ºC. Growth kinetics were performed in 100 mL YPD liquid medium with 10 g/L of yeast extract, 20 g/L of peptone and 10 g/L of glucose (autoclaved separately), and incubated overnight in an incubator at 30ºC at 200 rpm. A total of 10 samples were taken every hour and subsequent sampling at the 24-hour point. The parameters evaluated were OD at 600 nm (UV-2600 SHIMADZU), pH (QX1500 Qualxtron), and metabolite analysis by HPLC (Shimadzu, column Aminex HPX-87) of each sampling point (**Figure 1**). At the end of the cultivation, cell dry weight was also carried out by vacuum filtration and microwave drying cycles, for correlation with OD.

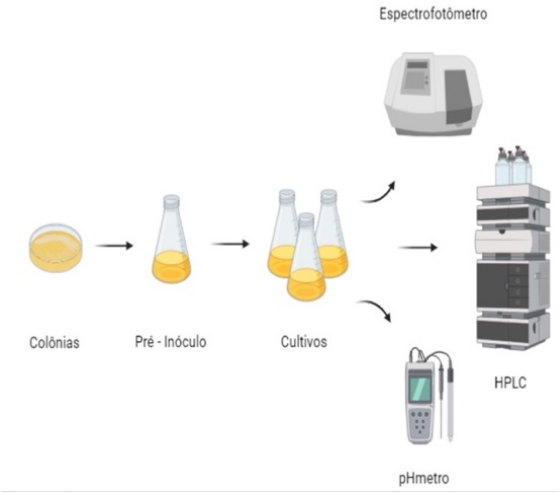

Figure 1. Flowchart of the *Y. lipolytica* strain cultivation process

3 RESULTS & DISCUSSION

When cultivated in a YPD with 4% glucose medium with the addition of 10% dodecane, BYa3105 strain was able to produce 35 ± 10 mg L^{-1} of geraniol, with a yield of 0.08 % C-mol geraniol (C-mol glucose)$^{-1}$ [7]. The growth kinetics parameters of *Y. lipolytica* modified strains BYa3105 and ST9202 and the parental strain W29 are shown in **Table 1**. The exponential phase of growth was identified as the linear region of the plot of ln (OD) versus time (**Figure 2b**), and the maximum specific growth rate (μ_{max}) was determined as the slope of this line. It was possible to calculate the generation time (Tg) by the quotient of ln (2) by μ_{max}. The substrate-to-cell conversion factor ($Y_{x/s}$) was determined as the slope of the line obtained by plotting the cell concentration (X g/L) as a function of the glucose depletion. Finally, the specific substrate consumption rate was derived by the quotient of μ_{max} by $Y_{X/S}$.[8]

Table 1 Physiological parameters of cultures with glucose as a the carbon source of *Y. lipolytica* strains W29, ST9202 and BYa3105.

Strain	X(g L^{-1})	Tg (h)	$Y_{X/S}$ (gX gS^{-1})	μ_{max} (h^{-1})	μ_s (gS gX^{-1} h^{-1})
W29	9,75	1,17	1,33	0,593	0,445
ST9202	7,54	1,34	1,24	0,517	0,415
BYa3105	6,10	1,36	1,02	0,509	0,500

Figure 2 illustrates the kinetics of growth (OD) and glucose consumption (a) and the exponential growth phase (b) of triplicate cultivations for each strain in YPD with 10 g/L glucose.

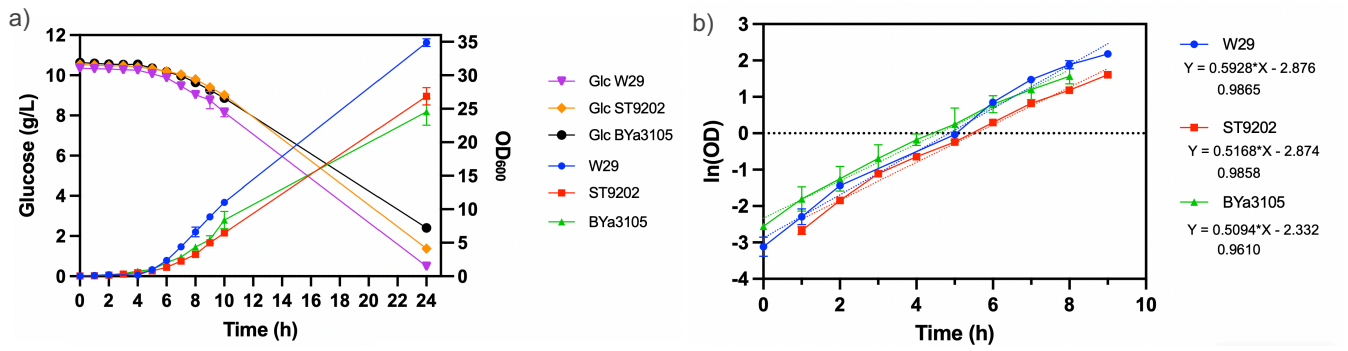

Figure 2 a) Glucose concentration (g/L) and OD at 600 nm during cultivations of triplicate cultivations for each strain in YPD with 10 g/L glucose, and b) the exponential growth phase

The parental strain W29, used as the control in this experiment, presented a substantial higher μ_{max} (0,593 h^{-1}) as compared to the modified strains. It also achieved a higher biomass concentration (9.75 g/L) at the end of the cultivation. Growth rates of strains ST9202 and BYa3105, were 13% and 14% lower than W29, respectively. The modified strains also presented a reduced biomass accumulation when compared to parental strain (Table 1).

Conducting a more thorough analysis, it is possible to infer that the redirection of metabolic resources towards terpene production likely contributed to the diminished growth kinetics observed in ST9202 and BYa3105. This metabolic shift likely introduced metabolic limitations through the activation of terpene biosynthetic pathways and the diversion of precursor molecules away from central metabolic processes. Additionally, as show in Table 1, BYa3105 had a comparable maximum specific growth rate (μ_{max}) to ST9202 despite presenting a slightly reduced total biomass accumulation at 24h. This could indicate that the insertion of the gene *GES* from Catharanthus roseus in BYa3105 is functional, as it does not affect the central metabolism and growth rate, showing no signs of eventual metabolic burden in addition to ST9202.

Regarding glucose consumption, strains W29 and ST9202 have comparable specific rate of substrate consumption (μ_S), 0,445 and 0,415 gS gX^{-1} h^{-1}, respectively. On the other hand, BYa3105 presented the highest specific rate of substrate consumption (0,500 gX gS^{-1}), with might be an indicative of carbon diversion to geraniol production or due to the toxic effects of this molecule. The geraniol producing strain also showed the lowest biomass yield (1,02 gX gS^{-1}) among the evaluated strains.

Overall, the analysis of glucose consumption rates reveals further information the metabolic activity of the yeast strains. BYa3105 demonstrated a high specific rate of glucose consumption, indicating its elevated metabolic activity, despite an inefficient utilization of resources for growth and metabolism. In contrast, ST9202 and W29 showed comparable specific rate of glucose consumption, despite ST9202 having slower growth kinetics suggesting a slight metabolic shift towards terpene biosynthesis. Therefore, this shows a tendency to impede overall growth progression shifting its metabolism from the cell to the production of geraniol, while ST9202 still maintains most of its metabolism focused on the production of biomass. In addition, as geraniol has shown anti-microbial properties [2], the lack biomass accumulation for the BYa3105 strain can be explained by the production of the monoterpene inhibiting cellular growth and biomass yield.

4 CONCLUSION

In conclusion, the observed differences in growth kinetics between W29 and the terpene-modified strains (ST9202 and BYa3105) highlight the relevant relationship between growth rate, biomass accumulation and terpene production. It is evident that genetic modifications can trigger metabolic exchanges when aimed at enhancing terpene biosynthesis, thus compromising overall biomass formation. The availability of crucial precursors for biomass accumulation and cellular development, can be diminished by the diversion of metabolic resources towards terpene synthesis and overall slow growth rates. However, it is important to point out that expression of the geraniol synthase gene from *C. roseus* in *Y. lipolytica* do not compromise important physiological parameters, such as growth rate.

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