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Influences of environmental factors on *Lactobacillus reuteri* growth and 3-hydroxypropionic acid production in the context of coupling with extraction

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Highlights

- Glycerol bioconversion into 3-hydoxypropionic by *Lactobacilllus reuteri*.
- Plackett – Burman method for screening factors.
- Influences of growth conditions on 3-hydroxypropionic production.

1. Introduction

In the framework of bioeconomy, 3-hydroxypropionic acid (3-HP) is gaining more and more interest due to its desirable industrial applications. As a bifunctionality molecule, 3-HP can be converted through chemical reactions into a variety of added-value chemicals, such as 1,3-propanediol (1,3-PDO), acrylic acid, malonic acid, or biodegradable polyesters [Matsakas et al., 2018], all of them give versatile applications in both industry and in everyday life. Among non-GMO microorganisms able to naturally produce 3-HP, *L. reuteri* is noticeable thanks to its ability to produce 3-HP from glycerol, which is a by-product in biodiesel manufacturing industries. This Gram-positive probiotic bacteria is an interesting candidate due to its G.R.A.S. status and ability to synthesize vitamin B12, an essential co-factor for the first enzyme of the bioconversion pathway. Having the ability to convert glycerol into equimolar concentration of 3-HP and 1,3-propanediol, but the cells do not use glycerol as a carbon source, thus avoiding the synthesis of by-products that can hinder extraction performance. However, the effects of nutritional and environmental factors during the growth phase, on the cell ability to perform bioconversion have not been elucidated yet. In this context, the aim of this work is to better understand the effect of these factors on the growth and the bioconversion, in order to identify the conditions to optimize the 3-HP production by *L. reuteri.*

2. Methods

2.1. The 3-stages process of 3-HP production by *L. reuteri.*

L. reuteri DSM 17938 that was purchased from BioGaia AB (Stockholm, Sweden) was cultivated in batch mode, in a controlled 5L bioreactor until reach to stationary phase. Cells then were harvested by centrifugation at 7500 rpm before being introduced into a 2L bioreactor working in fed-batch mode with glycerol addition at 0.5 $g.h^{-1}$. The cell concentration as quantified by flow cytometry (FCM), cell physiological states (determined by FCM coupled with carboxyfluorescein diacetate and propidium iodide staining [Rault *et al*. 2007]), substrate and metabolite products (quantified by liquid chromatography – HPLC [Burgé et al., 2015a].

2.3. Experimental design by Plackett-Burman method.

A Plackett-Burman experimental matrix was designed to test the effects of 11 growth factors on the growth phase and bioconversion ability of *L. reuteri* for 3-HP production [Plackett and Burman, 1946]. The tested factors include addition of glucose, yeast extract, phytone peptone, 1,2-propanediol (1,2-PDO), cysteine, betain plus KCl, tween 80, vitamin B12, lower pH at 5.5, lower temperature at 33°C. The results allow defining model of the following general form: $Y_i = k_i + \Sigma a_i \cdot Y_{(+)i}$ (Y_i : Response variable; k_i : Constant corresponding to the value of Y_i when all 11 variables are at their low level; a_i : Linear coefficient of each variable to express the effect of the high level; $Y_{(+)i}$: Value of the plus level of the variable) [Plackett and Burman, 1946]. All statically analysis for data were performed by the software Statgraphic plus version 5.0, at 95% confidence intervals for coefficient estimates.

3. Results and discussion

3.1. Effects of environmental factors on the cell growth.

The results showed that increasing glucose concentration into MRS medium gives a higher final cell concentration, together with a higher number of viable cells. Meanwhile, lower temperature of cultivation results in less growth and less acid lactic production, compared to the condition at 37°C.

3.2. Effects of environmental factors on cell ability to perform bioconversion.

By increasing glucose concentration, the molar ratio between 3-HP/1,3-PDO goes down. This can be explained that the more NADH produced during the glycolysis step are preferentially reoxidized to NAD^+ by the reduction of 3-HPA to 1,3-propanediol. Temperature decreases at 33°C gives no considerable effect since the P value is higher than 0.05. Surprisingly, the addition of vitamin B12 gives a negative impact to 3-HP production. A supplementary of such element in bioconversion stage under the presence of the substrate glycerol may give a better influence. Regarding to the duration of glycerol consumption, some interesting factors were observed, including the addition of betain and KCl, 1,2-PDO, and phytone peptone. It seems match to the previous studies that supplementary of betain at 2mM to medium consequence in the increase the cell tolerance under osmotic stress to *L. plantarum* [Ket et al. 1994]. Meanwhile, the presence of 1,2-propanediol is a preparation step for cell in the expression of the genes encoding the enzymes and structural proteins involving the *Pdu* pathway [Dishisha 2015a].

3.3. Conditions leading to the highest 3-HP production

The experimental design allowed to identify the best conditions that lead to the highest production of 3-HP at 19.6 g (corresponding to 15 $g.L^{-1}$), compared to the production of 13 g (12 $g.L^{-1}$) in the reference condition.

4. Conclusions

Addition of glucose increases cell proliferation but also decreases the molar ratio 3-HP/1,3-PDO. Conversely, lower temperature reduce the growth but gives no impact to bioconversion. Addition of vitamin B12 in the growth phase is not effective to 3-HP production while the presence of betain plus KCl, 1,2-PDO and phytone peptone extend the time of glycerol consumption. The selective factors giving positive impacts to the bioconversion will be considered in a second experimental design to optimize the conditions during the bioconversion step for 3-HP production by *L. reuteri.*

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