BIOPROCESS ENGINEERING

COMPARING THE KINETICS OF RHAMNOLIPIDS PRODUCTION BY *PSEUDOMONAS AERUGINOSA* LFM634 AND RHLC MUTANT

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ABSTRACT

Pseudomonas aeruginosa is capable of producing rhamnolipids, a biosurfactant of great commercial interest. The objective of this work was to compare the production of rhamnolipids by wild-type LFM634 and its *ΔrhlC* mutant, which produces exclusively monorhamnolipids, known for having superior surfactant properties. Experiments were done in benchtop bioreactors to evaluate the kinetics of cell growth and product formation, using the wild strain *Pseudomonas aeruginosa* LFM634 and a *ΔrhlC* mutant, which produces only monorhamnolipids. Cell growth was similar between the strains and the formation of rhamnolipids was slightly lower in the mutant (7.1 g/L) than the wild-type strain (8.9 g/L).

Keywords: Bioprocess. Biosurfactants. Rhamnolipids.

1 INTRODUCTION

Rhamnolipids (RL) are biosurfactants belonging to the class of glycolipids. They contain one or two rhamnose molecules in the polar portion and one or two lipid chains of 3-hydroxyalkanoates (3HA). Rhamnolipids can also present 3HA chains not chemically linked to rhamnose.[1][2]

Rhamnolipids are more efficient than synthetic surfactants, due to their chemical structure. The main one is related to the critical micelle concentration (CMC). For rhamnolipids, the CMC is lower than for synthetic surfactants, which means that they cause greater reductions in surface tension with the formation of micelles at lower concentrations.[3][4]

Some surfactant properties behave in different ways depending on the rhamnolipid chemical composition and structure. Compared to dirhamnolipids, monorhamnolipids showed lower surface tension (26.40 mN/m versus 32.04 mN/m), interfacial tension (1.14 mN/m versus 3.38 nM/m) and CMC (27.04 mg/L versus 90.58 mg/L). They also give a higher emulsification index (EI24 100% versus EI24 65%) and a better toxicity index (454 mg/L versus 156 mg/L), which puts them ahead for different applications, such as microbial enhanced oil recovery (MEOR) and antibiofilm effect.[5][6]

Rhamnolipids are synthesized by the bacterium *Pseudomonas aeruginosa*. This biosynthesis process occurs in three reactions in sequence. First, the acyltransferase RhlA catalyzes an ester bond between two 3-hydroxyalcyl-ACP molecules to form 3-(3-hydroxyalkanoyloxy)alkanoate (3HAA). Then, the rhamnosyltransferase I, or RhlB, catalyzes the connection of this lipid moiety to a molecule of (dTDP)-L-rhamnose, via a glycosidic O bond, thus forming a monorhamnolipid. Finally, rhamnosyltransferase II (RhlC) adds another molecule of (dTDP)-L-rhamnose to the monorhamnolipid through an α-1,2-glycosidic bond, giving rise to a dirhamnolipid.[7]

The objective of this research was to evaluate the kinetics of rhamnolipid formation by the wild-type LFM634 strain and its *ΔrhlC* mutant, which produces only monorhamnolipids.

2 MATERIAL & METHODS

Pseudomonas aeruginosa LFM634 (wild-type) and *ΔrhlC* mutant cells stored in 20% (v/v) glycerol at -80 °C were streaked to Lysogeny Broth (LB) agar for colony development and incubated for 48 to 72 h at 30 °C. From that, colonies were cultivated in liquid LB medium in 250 mL Erlenmeyer flasks using a medium volume/flask volume ratio of 20%, lasting 8 h, at 30 °C and under shaking at 150 rpm on a rotary shaker. The inoculum was grown in mineral medium, using a volume of 10% of pre-inoculum, maintaining medium volume/flask volume ratio of 20% in a 1 L Erlenmeyer flask, lasting 16 h, at 30 °C and shaking at 150 rpm on a rotary shaker. For cultivation in the bioreactor (EZ Control – Applikon Biotechnology - 6000 mL), 200 mL of the inoculum prepared as described above was inoculated in 2000 mL of medium. The cultivation was carried out at 30 °C and the pH was maintained at 7.00 ± 0.05, using 2.0 M NaOH or H_2SO_4. To avoid foam formation, only a surface aeration was carried out.

The samples were centrifuged (10000 g, 10 min, 4°C). Cell dry weight (CDW) was quantified by gravimetry of the freeze-dried pellet, glucose and rhamnose were quantified by high-performance liquid chromatography (HPLC) and polyhydroxyalkanoates (PHA) and 3HAA were quantified by gas chromatography (GC) of propyl esters. Residual Cell dry weight (RCDW) was calculated by subtracting the PHA concentration from the CDW concentration. The amount of rhamnolipids was given by the sum of the concentrations of rhamnose and 3HAA.

3 RESULTS & DISCUSSION

Both cultures showed maximum RCDW concentration after 18 h of cultivation, with the wild-type strain reaching 3.8 g/L, while the mutant reached 3.4 g/L (Figure 1). It was expected that the mineral medium provided would guarantee that the cellular concentration reached a maximum value of 5 g/L, and the main hypothesis regarding this non-occurrence is that part of the sodium nitrate used as a nitrogen source was directed to cellular respiration, since it is known that *Pseudomonas aeruginosa* is capable of that.[8] There was strong oxygen limitation in the medium around 12 h of cultivation, due to limited oxygen transfer by surface aeration, which reinforces the previous hypothesis. Both bacterial strains showed similar PHA accumulation, reaching about 0.8 g/L.
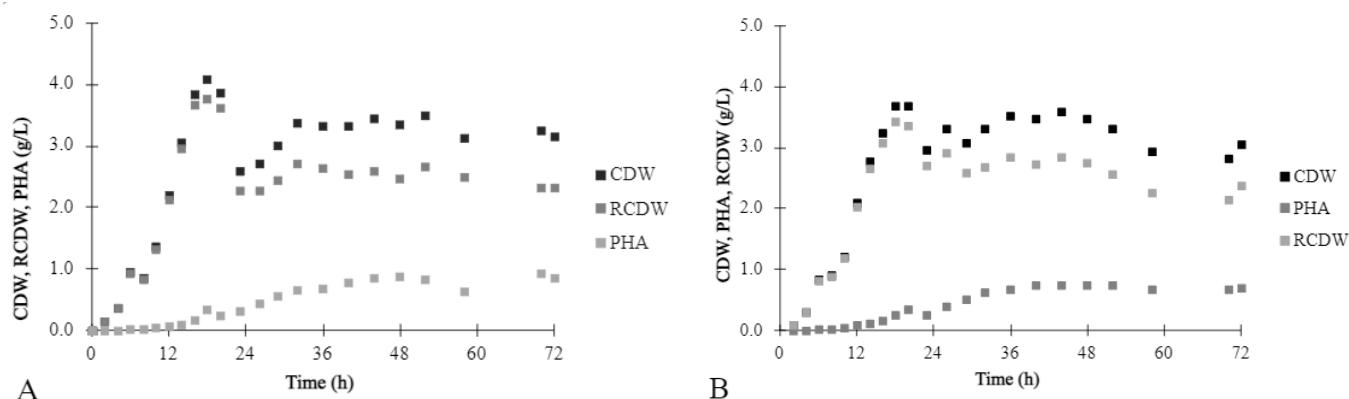

Figure 1 Profile of CDW, RCDW and PHA production in cultures (A) with wild-type *Pseudomonas aeruginosa* LFM634 and (B) with the *ΔrhlC* mutant for 72 h in bioreactors

The exponential growth phase for both cultures, determined by linear regression, occurred between 2 and 12 h, and at this moment, the maximum specific growth rate (μ_{max}) was 0.26 h^{-1} for the wild-type strain and 0.30 h^{-1} for the *ΔrhlC* mutant. These values are consistent with cultures under similar conditions, in the order of 0.27 h^{-1}, and may be higher in conditions with bubbling aeration, for example, reaching 0.31 to 0.37 h^{-1}.[7][9] After exponential growth phase, due to respiratory limitation, the growth was linear until 18 h of culture.

The yield coefficient, $Y_{X/S}$, was 0.16 g/g for both wild-type and for the mutant strain.

Regarding product formation, for the experiment using the wild-type strain, the final concentration of rhamnolipids was 8.9 g/L and for the mutant it was 7.1 g/L. This difference, of the order of 20%, was smaller in the bioreactor tests than that found in the preliminary shake flask experiments.

The rhamnolipid formation profiles for the cultures were similar (Figure 2), presenting three distinct phases: one during the exponential growth phase (up to 12 h of cultivation), in which there was no significant product formation, one during the linear phase and the first hours of the stationary phase (from 12 to 26 h), in which product release began, and finally in the remaining hours of the experiment (from 26 to 72 h), during the stationary phase, when there was a large amount of product formation.
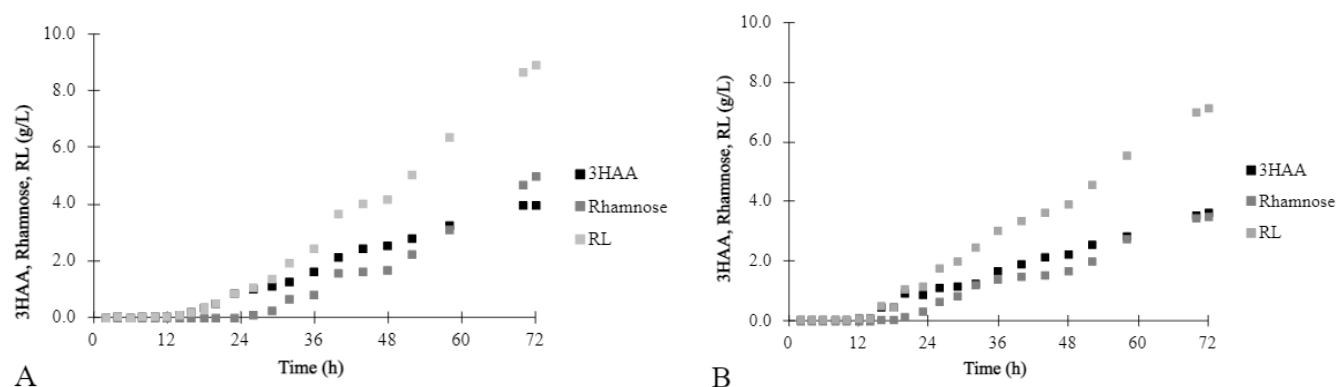

Figure 2 Profile of 3HAA, Rhamnose and RL production in cultures (A) with wild-type *Pseudomonas aeruginosa* LFM634 and (B) with the *ΔrhlC* mutant for 72 h in bioreactors

The substrate to product conversion factor, $Y_{P/S}$, values were similar for the two strains, 0.15 g/g. Finally, the overall volumetric productivity for the experiment with the wild-type strain was 0.12 g/(L.h) and for the mutant it was 0.10 g/(L.h). These values are within the range found in the literature for strains producing rhamnolipids under different cultivation conditions, reaching values such as 0.021, 0.0443 and 0.1491 g/(L.h). [7][9][10]

4 CONCLUSION

The *ΔrhlC* mutant strain presents cell growth and PHA accumulation similar to the wild-type strain of *Pseudomonas aeruginosa* LFM634, which is interesting for scaling-up of cultures in bioreactors. Rhamnolipid formation, however, was reduced in the mutant strain. Still, the promising properties of monorhamnolipids make their production of interest.

REFERENCES

[1]   SILVA, V. L. Estudo do metabolismo dos ramnolipídios produzidos por *Pseudomonas aeruginosa*. Thesis (Postgraduation in Biological Sciences – Applied Microbiology), Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP. Rio Claro. 2018.

[2]   ALMEIDA, K. L. Expressão heteróloga de genes rhlA envolvidos na síntese de 3-(3- hidroxialcanoiloxi)-alcanoato, o precursor de ramnolipídios. Thesis (Postgraduation in Microbiology). Universidade de São Paulo – USP. São Paulo. 2018.

[3]   BUENO, S. M. Bactérias produtoras de biossurfactantes: isolamento, produção, caracterização e comportamento num sistema modelo. 2008. 86 f. Thesis (Postgraduation in Biosciences), Universidade Estadual Paulista (UNESP). São Paulo. 2008.

[4]   NITSCHKE, M., PASTORE, G. M. Química Nova. 25 (5). 772-776. São Paulo. 2002.

[5]   ROCHA, V. A. L., CASTILHO, L. V. A. CASTRO, R. P. V., TEIXEIRA, D. B., MAGALHÃES, A. V., GOMEZ, J. G. C., FREIRE, D. M. G. Biotechnol Prog. 36(4). 2020.

[6]   ROCHA, V. A. L., CASTILHO, L. V. A., CASTRO, R. P. V., TEIXEIRA, D. B., MAGALHÃES, A. V., ABREU, F. A., CYPRIANO, J. B. S., GOMEZ, J. G. C., FREIRE, D. M. G. Biotechnol Prog. 37(3). 2021.

[7]   LOPÉZ-CHÁVEZ, S. A. Análise dos efeitos de diferentes limitações nutricionais na produção de ramnolipídios por Pseudomonas aeruginosa. Thesis (Postgraduation in Biotechnology). Universidade de São Paulo – USP, Instituto Butantan – IB, Instituto de Pesquisas Tecnológicas – IPT. São Paulo. 2019.

[8]   TOYOFUKU, M., O'TOOLE, G. A., NOMURA, N. Anaerobic life style of *Pseudomonas aeruginosa*. In: RAMOS, J.-L., GOLDBERG, J. B., FILLOUX, A. (Ed.). Pseudomonas. Dordrecht: Springer Science+Business Media. 7: New Aspp. 99–117. 2015.

[9]   ARAÚJO, J. S. Produção de ramnolipídeos por Pseudomonas aeruginosa AP029-GLVIIA usando glicose como substrato e aplicações. Thesis (Postgraduation in Chemical Engineering) – Universidade Federal do Rio Grande do Norte (UFRN). Natal. 2018.

[10]   KRONEMBERGER, F. A. Produção de Ramnolipídeos por Pseudomonas aeruginosa PA1 em Biorreator com Oxigenação por Contactor de Membranas. Thesis (Postgraduation in Chemical Engineering), Universidade Federal do Rio de Janeiro (UFRJ). Rio de Janeiro. 2007.