ENGINEERING A PROGRAMMED LYSIS SYSTEM FOR *Bacillus subtilis*

Gabriela B. de Paiva[1*] & Danielle B. Prdrolli[1]

[1]*Department of Bioprocess Engineering and Biotechnology, School of Pharmaceutical Sciences, UNESP- São Paulo State University, Araraquara, Brazil.*
** gabrielabpaiva@outlook.com*

ABSTRACT

Despite the intense growth of the market for biological compounds, their production and purification processes are still expensive. Therefore, to reduce the costs of the bioprocesses regarding the biocompounds, in this work we proposed the development of a production platform using *B. subtilis* as a chassis. The platform will accumulate the biocompound intracellularly, and release it to the extracellular environment after the production process is completed. To this end, we are engineering and testing a Programmed Lysis System to promote bacterial lysis triggered by carbon source depletion. The system is based on the Carbon Catabolite Repression and the sfGFP is a reporter for the system's validation. Preliminary results showed that the system is functional and able to promote *B. subtilis* lysis and release of the intracellular GFP without affecting normal growth and production yield.

Keywords: Programmed Lysis. Biocompounds. Synthetic Biology. *Bacillus subtilis*.

1 INTRODUCTION

Biocompounds are structurally complex molecules produced by living organisms[1]. Some of them are biopharmaceuticals with high cost and low market availability [1]. The market for biopharmaceuticals is constantly growing in the whole World. However, the costs for production and purification are high, especially due to the high purification yields required [2]. Within this context, we proposed the development of a platform for the production of biopharmaceuticals by engineering a bacterium to generate high product yields and release it to the extracellular environment. To this end, we chose the *B. subtilis* as a chassis. *B. subtilis* is a Gram-positive bacterium, classified as GRAS (Generally Recognized as Safe), able to produce high amounts of proteins, and is easily adaptable to large-scale cultivation with low growth requirements[3]. In this project, we proposed the development of a Programmed Lysis System to promote the cell lyses after the preferential carbon source is exhausted, releasing the intracellular content. This system will eliminate the costs related to cell disruption for the biocompound extraction, facilitating product recovery.

2 MATERIAL & METHODS

To engineer the Programmed Lysis System (PLS) in *B. subtilis*, a Serine-Integrase transcription unit was integrated into the genome locus *amyE*. The Serine-Integrase transcription unit is under the control of the carbon catabolite repression system. A copy of the Green Fluorescent Protein gene (*gfp*) was also inserted in the same genome locus under the control of a constitutive promoter. The GFP is the reporter for the system functionality tests. In another genome locus, the *lacA*, we inserted an Operon encoding two lytic enzymes and a constitutive promoter. The two elements are coded in different strands, and the two genes are flanked by recombination sites. Bacterial cultivations were carried out in a 24-deep-well microplate filled with 800 µL of Lysogeny Broth (LB) supplemented with 3% (m/v) glucose and incubated at 37ºC and 650 rpm. In addition, the strains were cultivated in LB supplemented with 3% (m/v) xylose under the same conditions. To monitor the PLS activation, the supernatant and cells were separated through centrifugation, and fluorescence was measured (excitation at 485 nm, and emission at 535 nm) and compared to the negative control[5]. Growth was measured spectrophotometrically at 600nm (OD_{600}) after the cells were resuspended in 1% (m/v) saline. To measure the fluorescence of the cells, the OD_{600} of the saline cell suspension was standardized to a similar value.

3 RESULTS & DISCUSSION

The Programmable Lysis System (PLS) is based on the Carbon Catabolite Repression (CCR), which means when the preferential carbon source is exhausted the bacteria will produce two enzymes that will promote the cell lysis[4]. As a reporter, we chose the super fold Green Fluorescent Protein (sfGFP) because it makes it possible to test the system's functionality through the intra- and extracellular fluorescence. When glucose is abundant, the CCR transcription factor represses the Serine-Integrase synthesis. As the glucose ceases, the transcriptional factor is released and the Serine-Integrase is synthesized. The enzyme should recombine the *attB* and *attP* sites inverting the Operon sense and activating the lytic enzymes synthesis. To test the PLS functionality, four colonies of the engineered *B. subtilis* containing the complete Programmable Lysis System were selected. In addition, as a negative control, a *B. subtilis* strain carrying an incomplete Programmable Lysis System (IPLS) was used.

The cells fluorescence remained constant throughout the cultivation of the strain IPLS and the four colonies of the strain PLS in both glucose (Figure 1-a) and xylose (Figure 1-b). Furthermore, fluorescence/OD_{600} values were similar between cultures with both carbon sources. Therefore, considering that both strains produce sfGFP, as well as both use glucose and xylose as a carbon source, and the gene expression is constitutive, it is not a surprise that the cells presented constant fluorescence.

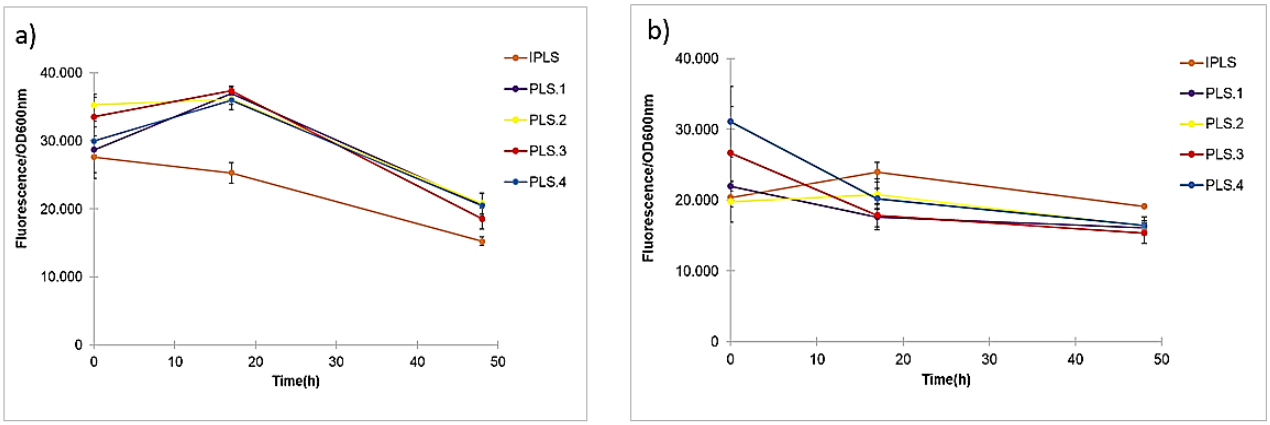

Figure 1 GFP fluorescence measured from cells. Cells fluorescence/OD_{600} of the strains IPLS and PLS grown in LB supplemented with 3% (m/v) glucose (a) and 3% (m/v) xylose (b).

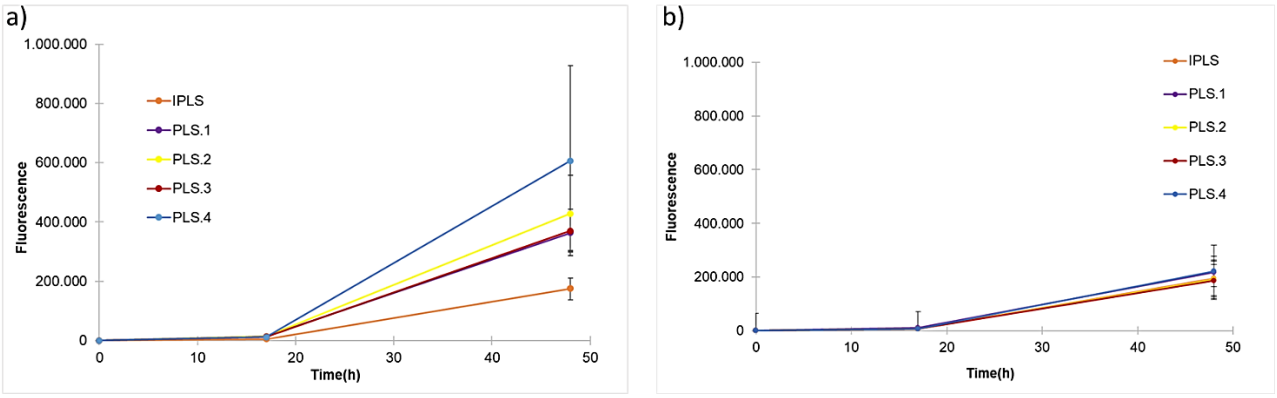

Figure 2 Supernatant's fluorescence of the strains IPLS and PLS grown in LB supplemented with 3% (m/v) glucose (a) and 3% (m/v) xylose (b).

The fluorescence in the supernatant of strains IPLS and PLS supplemented with xylose (Figure 2-b) were very similar to each other. The result is unexpected since xylose is a secondary carbon source and was supposed to activate the system. However, it acted as a repressor[6]. On the other hand, when the strains were supplemented with glucose, the supernatants of the 4 PLS colonies showed higher values than those of the IPLS, indicating that the PLS was correctly activated (Figure 2-a). Furthermore, after 48h of cultivation with glucose, the supernatants of the PLS colonies showed higher fluorescence values than the respective cultures on xylose. These initial results indicate the Programmed Lysis System works, resulting in the release of sfGFP to the extracellular environment after 20h of cultivation. However, more tests with different cultivation media and longer incubation times are required to access the system functionality fully.

4 CONCLUSION

We engineered and tested a Programmable Lysis System to release GFP as a reporter biocompound. The system was designed to reduce the costs related to protein extraction once it eliminates the cell disruption step. The preliminary results indicate that the PLS is functional and caused the cell lysis late in the cultivation. More tests are still needed to optimize the process and to quantify the sugar consumption during the growth.

REFERENCES

[1] KESIK-BRODACKA, M. Progress in biopharmaceutical development. Appl. and Environ. Microbiol. 65 (3). 306-322.

[2] KHANAL, O. LENHOFF, A. Developments and opportunities in continuous biopharmaceutical manufacturing. MABS. 13 (1). 1-13.

[3] LIU, Y., LIU, L., LI, J., DU, G., CHEN, J. Synthetic Biology Toolbox and Chassis Development in *Bacillus subtilis*. CellPress Reviews. 1-15.

[4] GÖRKE, B., STÜLKE, J. Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. Nat Rev Microbiol. 6. 613-624.

[5] OVERKAMP, W., BEILHARZ, K., WEME, R. D. O., SOLOPOVA, A., KARSENS, H., KOVACS, A., KOK, J., KUIPERS, O. P., VEENING, J. W. Benchmarking various green fluorescent protein variants in *Bacillus subtilis*, *Streptococcus pneumoniae*, and *Lactococcus lactis* for live cell imaging. Appl. and Environ. Microbiol. 79 (20). 6481-6490.

[6] TAVARES, L. F., RIBEIRO, N. V., ZOCCA, V. F. B., CORRÊA, G. G., AMORIM, L. A. S., LINS, M. R. C. R., PEDROLLI, D. B. Preventing Production Escape Using an Engineered GlucoseInducible Genetic Circuit. ACS Synth. Biol. 12 (10). 3124-3130.

ACKNOWLEDGEMENTS

This work is supported by the São Paulo State Foundation (FAPESP) grant 2021/01284-9, and the National Council for Scientific and Technological Development (CNPq) grants 310023/2020-3 and 305324/2023-3.