3-4-2018

# Production, immobilization and synthesis of pharmacological derivatives of lipase B from Candida antarctica in Pichia pastoris

Julia Robert
*Federal University of Rio de Janeiro, Brazil*, juliarobert601@gmail.com

Evelin A. Manoel
*Universidade Federal do Rio de Janeiro*

José Carlos Pinto
*Universidade Federal do Rio de Janeiro*

Denise M. G. Freire
*Universidade Federal do Rio de Janeiro*, freire@iq.ufrj.br

Martina C. C. Pinto
*Universidade Federal do Rio de Janeiro*

***See next page for additional authors***

**Authors**
Julia Robert, Evelin A. Manoel, José Carlos Pinto, Denise M. G. Freire, Martina C. C. Pinto, Roberto Fernandez-Lafuente, and Francisco Valero

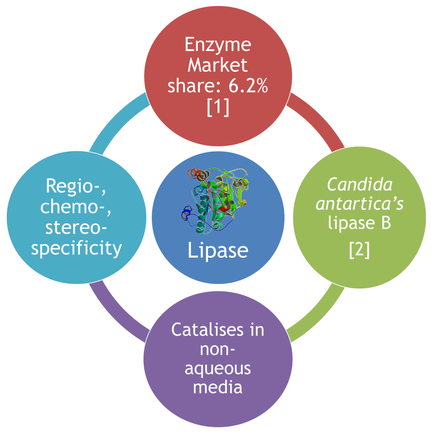
# PRODUCTION, IMMOBILIZATION AND SYNTHESIS OF PHARMACOLOGICAL DERIVATIVES OF LIPASE B FROM *CANDIDA ANTARCTICA* IN *PICHIA PASTORIS*


Julia de M. Robert[1*], Evelin A. Manoel[1], Martina C. C. Pinto[1], Roberto Fernandez-Lafuente[2], José Carlos Pinto[1], Francisco Valero Barranco[3] and Denise M. G. Freire[1*]

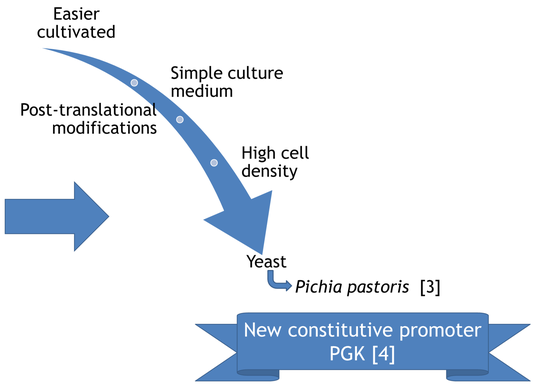
[1]*Universidade Federal do Rio de Janeiro, Brazil*
[2]*CISIC, Madrid*
[3]*Universitat Autònoma de Barcelona, Barcelona.*

*e-mail: juliarobert601@gmail.com ; freire@iq.ufrj.br


## Lipase interest

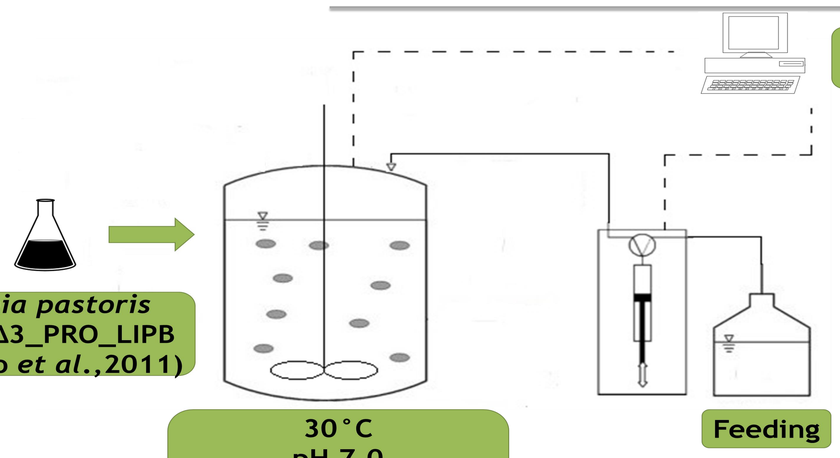



## Why *Pichia pastoris* as a host?



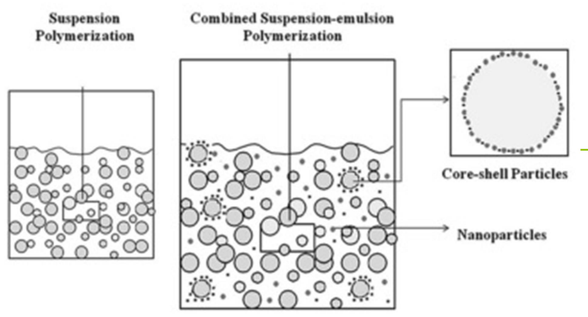

## METHODOLOGY

### Production




Open Loop

### Immobilization – no purification step needed

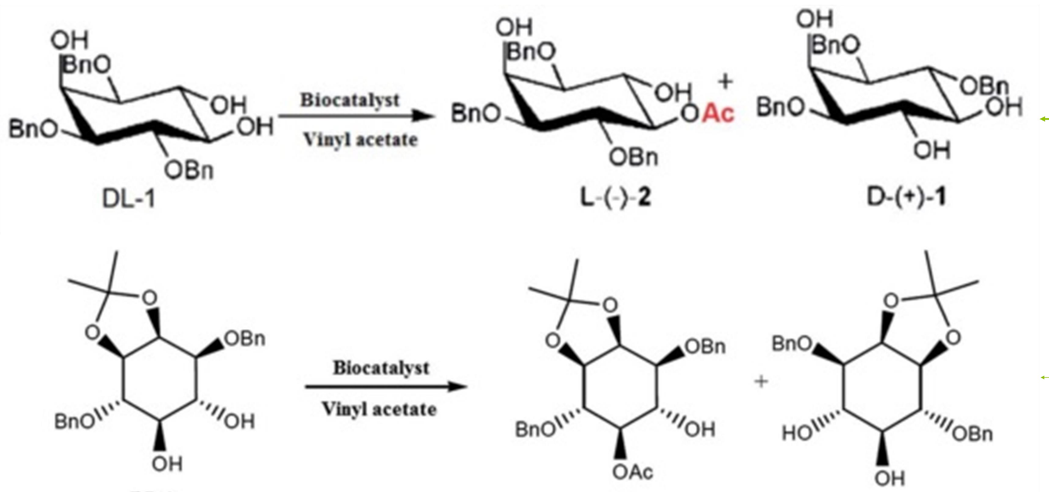



Immobilization

DL-1
8 mg subs
2 ml vinyl acetate
hexano (1:5,5)
45 °C
222,8 U

DL-2
10 mg subs
2 ml vinyl acetate
solv.free
45°C
71 U

### Synthesis




## RESULTS

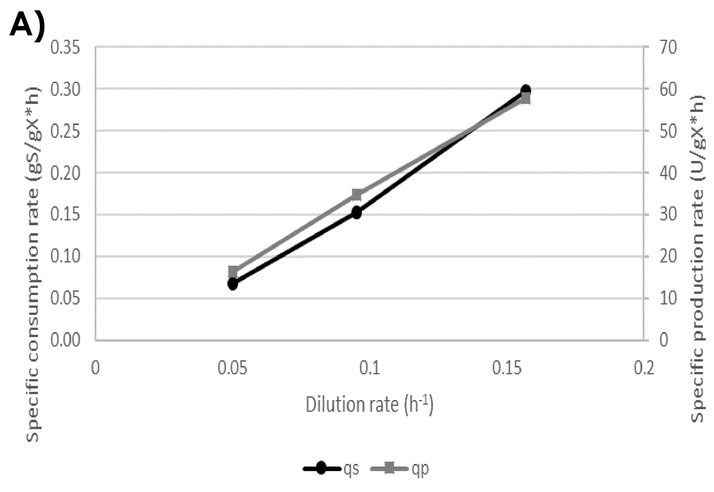



**Figure 1: Specific carbon source uptake rate and specific production rate determined in continuous (A) and fed-batch (B) culture for different specific growth rates of *Pichia pastoris* growing on glycerol.**

**Table 1: Results of racemic resolution of DL-1 with CALB and LIPB immobilized on different supports.**

| Commercial lipase | *X* (%) | $ee_p$ | *E* | Initial velocity (μmol $min^{-1}$ $g^{-1}$) × $10^2$ |
|---|---|---|---|---|
| Lyophilized CALB | $20^b \pm 0.1$ | 99 | >200 | 3.30 |
| CALB immobilized on Accurel MP 1000 | $40.6^b \pm 0.2$ | 99 | >200 | 6.71 |
| CALB immobilized on PS-*co*-DVB/PS-*co*-DVB | $42.0^b \pm 0.1$ | 99 | >200 | 6.94 |
| CALB immobilized on PMMA-*co*-DVB/PMMA-*co*-DVB | $40.0^b \pm 0.2$ | 99 | >200 | 6.61 |
| CALB immobilized on PMMA/PMMA | $31.0^b \pm 0.5$ | 99 | >200 | 3.21 |
| Novozyme 435 | $49.7^a \pm 0.1$ | 99 | >200 | 8.21 |
| **Recombinant lipase** | ***X* (%)** | **$ee_p$** | ***E*** | **Initial velocity (μmol $min^{-1}$ $g^{-1}$) × $10^2$** |
| Lyophilized LIPB | $3.0^b \pm 0.5$ | 99 | >200 | 0.74 |
| LIPB immobilized on Accurel MP 1000 | $41.0^a \pm 0.5$ | 99 | >200 | 10.16 |
| LIPB immobilized on PS-*co*-DVB/PS-*co*-DVB | $47.5^a \pm 0.1$ | 99 | >200 | 11.77 |
| LIPB immobilized on PMMA-*co*-DVB/PMMA-*co*-DVB | $41.2^a \pm 0.1$ | 99 | >200 | 10.21 |
| LIPB immobilized on PMMA/PMMA | $36.2^a \pm 0.1$ | 99 | >200 | 5.30 |

[a] Batch conditions: 45 °C; 4 mg $mL^{-1}$ of substrate; 222.8 U of enzyme; 8 h. Hexane as solvent. [b] 12 h reaction time.

**Table 2: Results of the enantioselective resolution of DL-2 for the different versions of *Candida antarctica* lipase B CALB.**

| CALB (commercial) | *X* (%) | $ee_p$ | *E* | Initial velocity (μmol $min^{-1}$ $g^{-1}$) × $10^2$ |
|---|---|---|---|---|
| Lyophilized CALB | $28.0^b \pm 0.8$ | 99 | >200 | 4.93 |
| CALB immobilized on Accurel MP 1000 | $40.8^b \pm 0.1$ | 99 | >200 | 7.18 |
| CALB immobilized on PS-*co*-DVB/PS-*co*-DVB | $45.3^a \pm 0.5$ | 99 | >200 | 7.97 |
| CALB immobilized on PMMA-*co*-DVB/PMMA-*co*-DVB | $43.3^a \pm 0.2$ | 99 | >200 | 7.62 |
| CALB immobilized on PMMA/PMMA | $33.0^b \pm 0.5$ | 99 | >200 | 4.21 |
| Novozyme 435 | $49.0^a \pm 0.1$ | 99 | >200 | 8.62 |
| **Recombinant lipase** | ***X* (%)** | **$ee_p$** | ***E*** | **Initial velocity (μmol $min^{-1}$ $g^{-1}$) × $10^2$** |
| Lyophilized LIPB | $18.0^b \pm 0.8$ | 99 | >200 | 4.75 |
| LIPB immobilized on Accurel MP 1000 | $48.0^a \pm 0.5$ | 99 | >200 | 12.67 |
| LIPB immobilized on PS-*co*-DVB/PS-*co*-DVB | $49.3^a \pm 0.5$ | 99 | >200 | 13.01 |
| LIPB immobilized on PMMA-*co*-DVB/PMMA-*co*-DVB | $48.6^a \pm 0.2$ | 99 | >200 | 12.83 |
| LIPB immobilized on PMMA/PMMA | $36.0^b \pm 0.5$ | 99 | >200 | 5.88 |

[a] Batch conditions: 45 °C; 5 mg $mL^{-1}$ of substrate; 71 U of enzyme; 8 h reaction time; solvent-free reaction; vinyl acetate as acylating agent and solvent. [b] Batch conditions: 45 °C; 5 mg $mL^{-1}$ of substrate; 71 U of enzyme; 13 h reaction time; solvent-free reaction; vinyl acetate as acylating agent and solvent.

## CONCLUSIONS

- Higher $q_s$ achieved with continuous culture at μ=0.15$h^{-1}$ (57.71U/gX.h)
- PS-co-DVB most efficient in the enantioselective resolution of both racemic derivatives
- Productivity for DL-2 resolution with LIPB was 50% higher than the commercial Novozym 435, and the new derivative was operationally more stable.


## ACKNOWLEDGMENTS

GOBIERNO DE ESPAÑA · MINISTERIO DE EDUCACIÓN, CULTURA Y DEPORTE · CAPES · PETROBRAS


## REFERENCES


[1]Freedonia Group, World enzyme demand, 2014.
[2]Joseph, B., Ramteke, P.W., Thomas, G., Cold active microbial lipases: Some hot issues and recent developments. Biotechnol. Adv., 2008. 26: 457–470.
[3]Çelik, E., Çalık, P., Production of recombinant proteins by yeast cells. Biotechnol. Adv., 2012. 30: 1108–1118.
[4] Robert, J.M., Lattari, F.S., Machado, A.C., de Castro, A.M., Almeida, R.V., Torres, F.A.G., Production of recombinant lipase B from Candida antarctica in Pichia pastoris under control of the promoter PGK using crude glycerol from biodiesel production as carbon source. Biochem. Eng. J., 2017. 118: 123–131.
[5] Garcia-Ortega, X.; Ferrer, P.;Montesinos, J. L.; Valero, F., Fed-Batch operational strategies for recombinant Fab production with Pichia pastoris using the constitutive GAP promoter. Biochem. Eng. J. 2013. 79: 172-181.
[6] Manoel EA, Robert JM, Pinto MCC, Machado ACO, Besteti MD, Maria F, et al. Evaluation of the performance of differently immobilized recombinant lipase B from Candida antarctica preparations for the synthesis of pharmacological derivatives in organic media. RSC Adv. 2016;6:4043–52.