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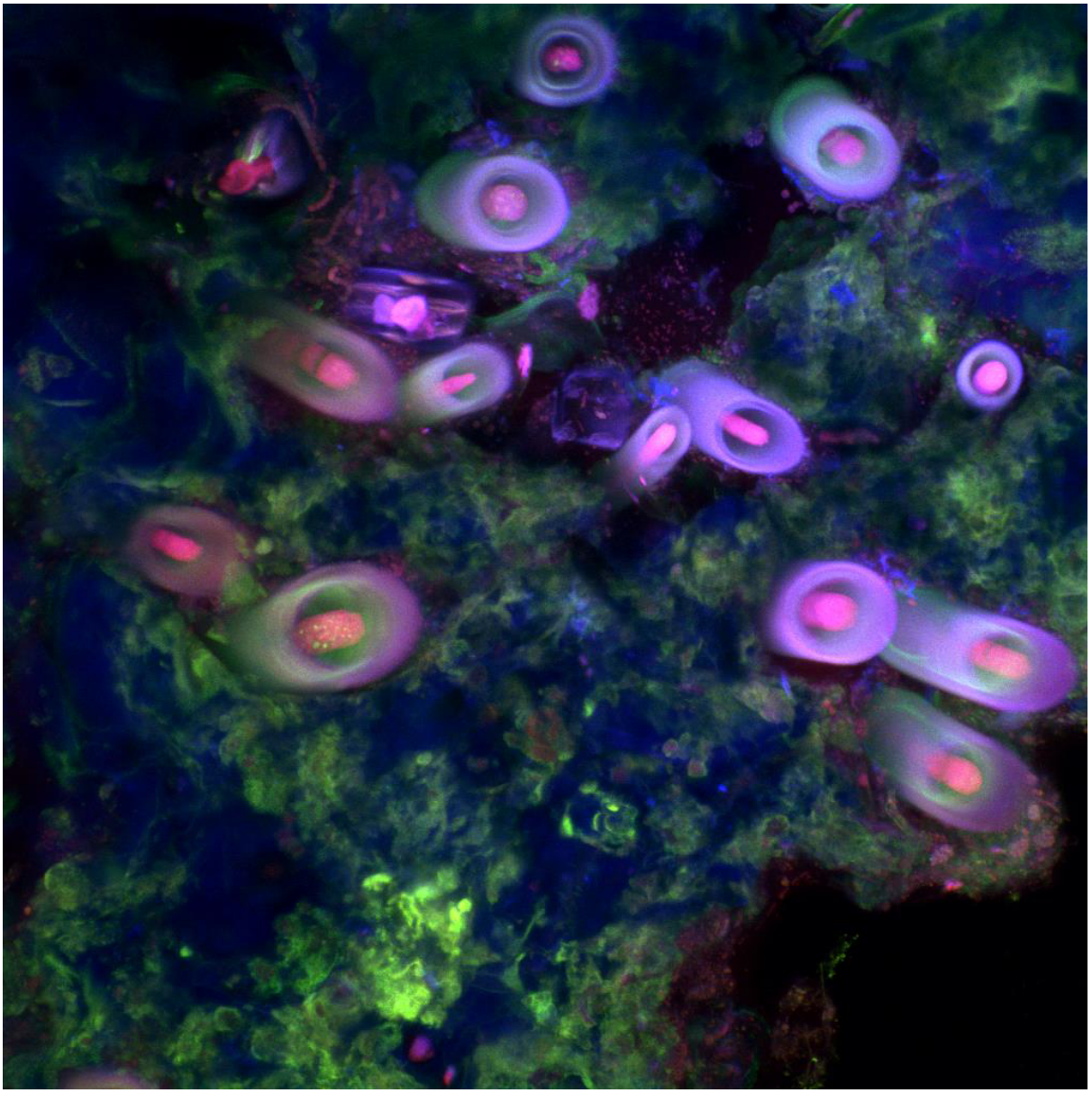
LVI SAIB Meeting – XV SAMIGE Meeting



SAIB-SAMIGE Joint Meeting 2020 – *Online*

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Cover image:

Mineral–microorganisms interactions

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A Confocal Laser Scanning Microscopy image of a resin-embedded microbialite from Laguna Negra (Puna-Catamarca), stained with calcein (a fluorescent dye that produces a stable complex in the presence of calcium and fluoresces in the green region of visible light). Mineral aggregates are observed in blue. Their surfaces are partially stained with calcein, indicate the presence of free Ca^{2+} ions. Diatoms and *Rivularia halophila* filaments are visible in red thanks to their photosynthetic pigments.

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LVI Annual Meeting
Argentine Society for Biochemistry and Molecular Biology
(SAIB)

XV Annual Meeting
Argentinean Society for General Microbiology
(SAMIGE)

SAIB-SAMIGE – Online
Joint Meeting 2020

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BT-C11-266
COMPARISON OF SARS-COV-2-SPIKE RECEPTOR BINDING DOMAIN PRODUCED IN *Pichia pastoris* AND MAMMALIAN CELLS

Argentinian AntiCovid Consortium*: formed by 30 researchers of CONICET working at iB3 and AGBT, FBMC, FCEN, UBA; QB, FCEN, UBA; FFyB, UBA; FMed, UBA; IIB-UNSAM, I. Milstein and UTN E-mail: anticovid.arg@gmail.com*

A few months ago, just before SARS-CoV-2 spread in Argentina, a group of researchers from different institutions and with diverse relevant expertise began a collaborative work to help fight the pandemic. Our work was aimed at producing at low cost the receptor-binding domain (RBD) of SARS-CoV-2 spike protein, as it is useful for serological detection of infected patients, as a selection antigen to obtain neutralizing antibodies in animal models, and as a vaccine candidate. The yeast *Pichia pastoris* is a cost-effective and easily scalable system for recombinant protein production. In this work, we compared the conformation of the RBD from SARS-CoV-2 Spike protein expressed in *P. pastoris* and in the well-established HEK-293T mammalian cell system. Our results showed that (i) RBD produced in both systems was properly folded; (ii) mass spectrometry analysis and glycosidase digestion suggested that both forms are differentially glycosylated; (iii) both variants are conformationally stable and their stabilities depend on the ionic strength in the same way, and (iv) antibodies generated in mice injected with proteins produced in yeast recognize the protein produced in mammalian cells and vice versa. The production of RBD in *P. pastoris* was scaled-up in a bioreactor, with yields above 60 mg/L of 90% pure protein, thus potentially allowing large scale immunizations to produce neutralizing antibodies, as well as the large-scale production of serological tests for SARS-CoV-2.

Argentinian AntiCovid Consortium (in alphabetical order with equal contribution of all authors): Claudia R. Arbeitman, Analía Amante, Gabriela Auge, Matías Blaustein, Luis Bredeston, Enrique S. Corapi, Patricio O. Craig, Leandro A. Cossio, Liliana Dain, Cecilia D'Alessio, Fernanda Elias, Natalia B. Fernández, Yamila Gándola, Javier Gasulla, Natalia Gorojovsky, Gustavo E. Gudesblat, María G. Herrera, Lorena I. Ibañez, Tommy Idrovo Hidalgo, Matías Iglesias Randon, Laura Kamenetzky, Alejandro D. Nadra, Diego G. Noseda, Carlos H. Paván, María F. Pavan, María F. Pignataro, Javier Ramirez, Ernesto Roman, Lucas A. M. Ruberto, Natalia Rubinstein, Javier Santos, Francisco Velazquez Duarte, Diana E. Wetzler and Alicia M. Zelada.

BT-C12-282
OPTIMIZATION OF PH FOR L-DOPA PRODUCTION IN BENCH-TOP SCALE STIRRED-TANK BIOREACTOR USING A *Paraboeremia* STRAIN

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Biosimilars contain active molecules from a biological source, have the same biological function but come from a manufacturing process, which differs from the traditional chemical production synthesis of the original drug, their active principles are usually more complex than those of the chemically synthesized counterparts, and only living organisms can reproduce this complexity. Parkinson is a disease associated with dopamine deficiency and levodopa has been the elected drug to treat it for decades. In recent years, various advances have allowed understanding the mechanisms of action. The objective of this work was to study the influence of initial pH in the L-Dopa microbiological production process, by means of the operation of a bench-top bioreactor, using the selected fungus *Paraboeremia* LY 38.7, isolated from Las Yungas Tucumanas. For the production of L-Dopa at bioreactor scale and to study the effect of initial pH, previously optimized conditions at smaller scale were used as starting point. Accordingly, the initial pH was varied between 5.0 and 7.5, keeping constant further operative conditions for 5 days, i.e., aeration (1 vvm), temperature (25°C) and stirring rate (200 rpm), and sampling twice a day. Production of L-Dopa and L-Tyrosine consumption were measured by means of the Arnow method, tyrosinase monophenolase and diphenolase activities according to the dopachrome method, biomass by dry weight estimation and pH with a pHmeter. Results showed that volumetric productivity of L-Dopa was higher at pH 7 and 7.5 (4.11 $mg \cdot L^{-1} \cdot h^{-1}$ and 3.65 $mg \cdot L^{-1} \cdot h^{-1}$ respectively). These optimal pH values represented favourable conditions from the operational point of view, taking into account that recently prepared culture medium has a pH of 7.5. Additionally, it is also convenient in terms of productivity, since maximal L-Dopa production is achieved one day earlier (48 instead of 72 h) than at pH 5.0, 5.5, 6.0, and 6.5, which implies lower operating costs and adds more economic and industrial attractiveness.