Less is more: Genome-reduced Bacillus subtilis for protein production

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Abbreviations

ADP	Adenosine diphosphate
AEC	Adenylate energy charge
AMP	Adenosine monophosphate
AUC	Area under the curve
ATP	Adenosine triphosphate
CHIPS	Chemotaxis inhibitory protein
DBT cycle	Design, Build, Test cycle
DCW	Dry cell weight
FDR	False discovery rate
HPLC	High-performance liquid chromatography
iBAQ	Intensity-based absolute quantification algorithm
IsaA	Immunodominant *Staphylococcus aureus* antigen A
KDa	Kilo Dalton
LB	Lysogenic broth
LC/MS	Liquid chromatography–mass spectrometry
LDS-PAGE	Lithium dodecyl sulfate-polyacrylamide gel electrophoresis
LFQ	Label-free quantification intensities
LTA	Lipoteichoic acid
MS	Mass Spectrometry
Nuc	Nuclease
PBPs	Penicillin binding proteins
PCA	Principal component analysis
PG	Peptidoglycan
PMM	Pumilus minimal media
OD_{600}	Optical density at 600 nm
SCIN	Staphylococcal complement inhibitor
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SMM	Spizizen minimal media
SRM	Selected Reaction Monitoring
TCA	Trichloroacetic acid
TE buffer	Tris EDTA buffer
TEAB buffer	Tetraethylammonium bromide buffer
TMD	Transmembrane domain
UPS2	Universal Proteomics Standard
$Y_{X/S}$	Biomass yields on substrate

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List of Publications

- Bernal-Cabas, M., Miethke, M., Antelo-Varela, M., **Aguilar Suárez, R.**, Neef, J., Schön, L., Gabarrini, G., Otto, A., Becher, D., Wolf, D., & van Dijl, J. M. (2020). Functional association of the stress-responsive LiaH protein and the minimal TatAyCy protein translocase in *Bacillus subtilis*. *Biochimica et Biophysica Acta - Molecular Cell Research, 1867(8), [118719].*

- Antelo-Varela, M., **Aguilar Suárez, R.**, Bartel, J., Bernal-Cabas, M., Stobernack, T., Sura, T., van Dijl, J. M., Maaß, S., & Becher, D. (2020). Membrane modulation of super-secreting 'midi*Bacillus*' expressing the major *Staphylococcus aureus* antigen -A Mass-Spectrometry-based absolute quantification approach. *Frontiers in Bioengineering and Biotechnology,* 8, [143].

- **Aguilar Suarez, R.**, Stülke, J., & van Dijl, J. (2019). Less is more: towards a genome-reduced *Bacillus* cell factory for 'difficult proteins'. *ACS Synthetic Biology*, 8(1), 99-108.

- Heuker, M., Sijbesma, J. W. A., **Aguilar Suárez, R.**, de Jong, J. R., Boersma, H. H., Luurtsema, G., Elsinga, P. H., Glaudemans, A. W. J. M., van Dam, G. M., van Dijl, J. M., Slart, R. H. J. A., & van Oosten, M. (2017). In vitro imaging of bacteria using (18)F-fluorodeoxyglucose micro positron emission tomography. *Scientific Reports*, 7, [4973].

- Mendoza-Martínez, C., Correa-Basurto, J., Nieto-Meneses, R., Márquez-Navarro, A., **Aguilar-Suárez, R.**, Montero-Cortes, M. D., Nogueda-Torres, B., Suárez-Contreras, E., Galindo-Sevilla, N., Rojas-Rojas, Á., Rodriguez-Lezama, A., & Hernández-Luis, F. (2015). Design, synthesis and biological evaluation of quinazoline derivatives as anti-trypanosomatid and anti-plasmodial agents. *European Journal of Medicinal Chemistry,* 96, 296-307.

Biography

Rocío Aguilar Suárez studied chemistry, bacteriology and microbiology at the National Polytechnic Institute in Mexico City. After the culmination of her bachelor's programme with a thesis she received her bachelor's degree with a distinction in 2011. The research described in her bachelor's thesis focused on the assessment of antimalarial activity of quinazoline derivatives in a murine model. In addition to her studies, she was also involved in policy activities. After her graduation, she did a research stay at the Center of Research and Advanced Studies, campus Merida in Mexico, where she worked with prof. Leopoldina Aguirre Macedo, and worked on petroleum hydrocarbon-degrading bacteria. Later, she moved to the Netherlands to pursue a master's degree in molecular biology and biotechnology at the University of Groningen. During her studies she joined the molecular genetics research group of prof. Oscar Kuipers at the Groningen Institute Biomolecular Sciences & Biotechnology, where she worked on lantibiotic production in *Lactococcus lactis*. Consecutively, Rocío joined to the Molecular bacteriology research group of prof. Jan Maarten van Dijl at the University Medical Center Groningen, where she worked on the role of a small regulatory RNA in *Bacillus subtilis*. After receiving her master's degree in 2014, she became a PhD candidate at the University of Groningen where she worked on the development of genome-reduced *Bacillus subtilis* strains for protein production under the guidance of prof. Jan Maarten van Dijl. During her PhD trajectory, she did a research stay at the Microbial Proteomics research group of prof. Dörte Becher at the University of Greifswald in Germany, followed by a research stay at the Institute for Systems biotechnology of prof. Christoph Wittmann at Saarland University in Germany. The results of this collaborative work are described in the present thesis. Besides her research activities, she participated in the policy activities as a member of the University Council of the University of Groningen.