



Università degli Studi di Napoli Federico II

PhD in Biotechnology - 40th cycle

Dr. Joan Bermejo i Cuadros

**Structural elucidation of archaeal
exopolysaccharides and identification of candidate
enzymes involved in glycans modification**

Tutors: Marco Moracci (BIOS-07/A), Andrea Strazzulli (BIOS-07/A)

Department: Dipartimento di Biologia, Edificio 7, Complesso Universitario Monte Sant'Angelo – Strada Vicinale Cupa Cintia, 21 – 80126, Napoli (Italia)

The extremophilic traits of archaea make them promising candidates for diverse biotechnological applications, including sustainable biofuel production, biochemical synthesis, and nanotechnology, contributing to a circular bioeconomy^{1,2,3}. However, critical aspects of their cellular and molecular biology remain poorly understood, limiting their full biotechnological potential⁴. One particularly important aspect is archaeal biofilm, which holds transformative applications across multiple industries, including infrastructure, food, and pharmaceutical^{5,6}. The biofilm matrix is primarily composed of proteins, nucleic acids, and exopolysaccharides (PS), with PS playing a crucial role in the stability and development of the biofilm⁷. While previous studies have characterized the monosaccharide composition of archaeal PS⁷, the detailed glycan linkages, including stereospecificity and regiospecificity, remain largely unexplored, hindering their biotechnological exploitation. For that reason, this PhD project aims to address this knowledge gap by employing glycoside hydrolases (GHs) to analyse glycan linkages in archaeal PS, complemented by physicochemical methods for detailed structural characterization. Available GHs will be utilized to systematically degrade PS into oligosaccharide fragments, which will then be analysed using High-Performance Liquid Chromatography (HPLC), to elucidate their structure and glycan linkages. The feasibility of this approach has been previously demonstrated in our laboratory^{8,9}. However, given the potential limitations of existing GHs in recognizing all sugar residues and glycosidic bonds in archaeal PS, this project will also focus on identifying and characterizing novel enzymes for biofilm hydrolysis, thus expanding the available enzymatic toolkit for PS analysis.

References

1. Marcellin, E., Angenent, L. T., Nielsen, L. K., & Molitor, B. (2022). Recycling carbon for sustainable protein production using gas fermentation. *Current Opinion in Biotechnology*, *76*, 102723.
2. Moissl, C., Rachel, R., Briegel, A., Engelhardt, H., & Huber, R. (2005). The unique structure of archaeal 'hami', highly complex cell appendages with nano-grappling hooks. *Molecular microbiology*, *56*(2), 361-370.
3. Sitara, A., Hocq, R., Horvath, J., & Pflügl, S. (2024). Industrial biotechnology goes thermophilic: Thermoanaerobes as promising hosts in the circular carbon economy. *Bioresource Technology*, 131164.
4. van Wolferen, M., & Albers, S. V. (2022). Progress and Challenges in Archaeal Cell Biology. *Archaea: Methods and Protocols*, 365-371.
5. Davidova, I. A., Duncan, K. E., Perez-Ibarra, B. M., & Suflita, J. M. (2012). Involvement of thermophilic archaea in the biocorrosion of oil pipelines. *Environmental microbiology*, *14*(7), 1762-1771.
6. Puiu, R. A., Dolete, G., Ene, A. M., Nicoară, B., Vlăsceanu, G. M., Holban, A. M., & Bolocan, A. (2017). Properties of biofilms developed on medical devices. In *Biofilms and Implantable Medical Devices* (pp. 25-46). Woodhead Publishing.
7. Kuschmierz, L., Meyer, M., Bräsen, C., Wingender, J., Schmitz, O. J., & Siebers, B. (2022). Exopolysaccharide composition and size in *Sulfolobus acidocaldarius* biofilms. *Frontiers in Microbiology*, *13*, 982745.
8. Briggs, D. C., Yoshida-Moriguchi, T., Zheng, T., Venzke, D., Anderson, M. E., Strazzulli, A., & Campbell, K. P. (2016). Structural basis of laminin binding to the LARGE glycans on dystroglycan. *Nature chemical biology*, *12*(10), 810-814.
9. Curci, N., Strazzulli, A., Iacono, R., De Lise, F., Maurelli, L., Di Fenza, M., & Moracci, M. (2021). Xyloglucan oligosaccharides hydrolysis by exo-acting glycoside hydrolases from hyperthermophilic microorganism *Saccharolobus solfataricus*. *International Journal of Molecular Sciences*, *22*(7), 3325.