Functional dissection of the large adhesion protein

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Introduction

There are numerous microorganisms which have the ability to switch between being in planktonic state or form biofilms, these biofilms are complex communities of microorganisms attached to surfaces or associated with interfaces (Figure 1). This concept has a lot of relevance in ecology due to the fact that these microbial communities are often composed of multiple species that interact with each other and their environment [1]. The longest gene in the Gram-negative bacterium *Pseudomonas putida* genome encodes LapA (Figure 2), a >9000 amino-acid surface adhesin essential to surface adhesion and biofilm formation. LapA is a complex protein, containing numerous functional domains and a large array of repeated sequences. However, the exact function of any of these elements in LapA is unknown [2].

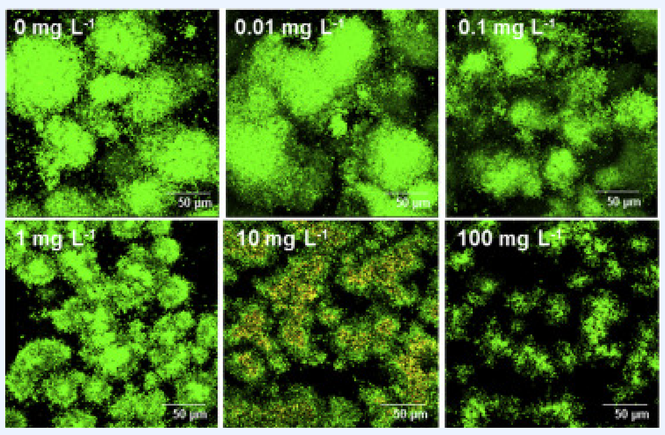

Figure 1. Qualitative characterisation of the *P. putida* biofilm morphology with different concentration of silver nanoparticles at 72 h. (Auerbach et al.,2000)

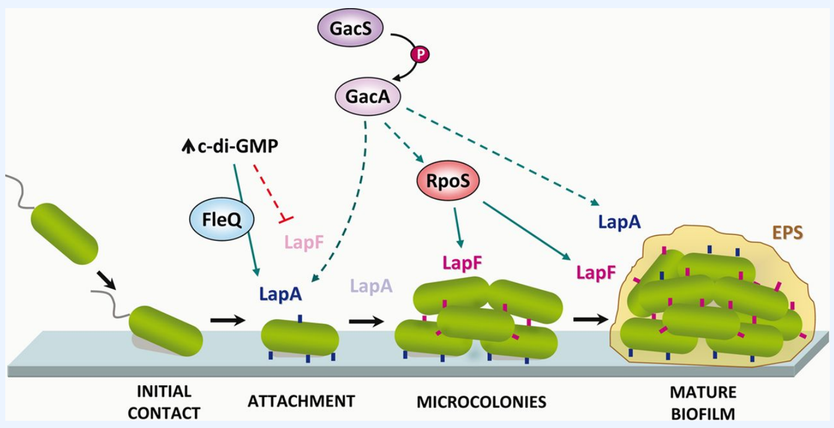

Figure 2. Role of LapA adhesin in biofilm formation. (Martínez-Gil, M. et al.,2014)

Objectives

- The construction of different lapA variants containing internal deletions of the putative functional domains
- The study the role of each of these in LapA-dependent phenotypes.

Experimental design

Each version will be inserted in the chromosome of a ΔlapA mutant using a Tn7-based delivery system. An Initial synthetic construct containing the complete N-terminal and C-terminal domains and a 3xHA tag for immunodetection, but lacking all repeated sequences is already available (Figure 3), and constructs bearing progressively shorter N-terminal domains are underway (Figure 4). Addition of different numbers of repeats will be tested afterwards.

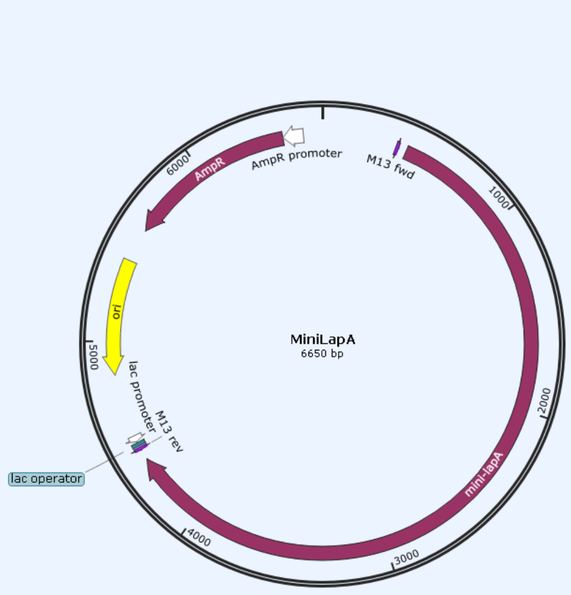

Figure 3. Synthetic construct containing the complete N-terminal and C-terminal domains and a 3xHA tag for immunodetection without repeated sequences

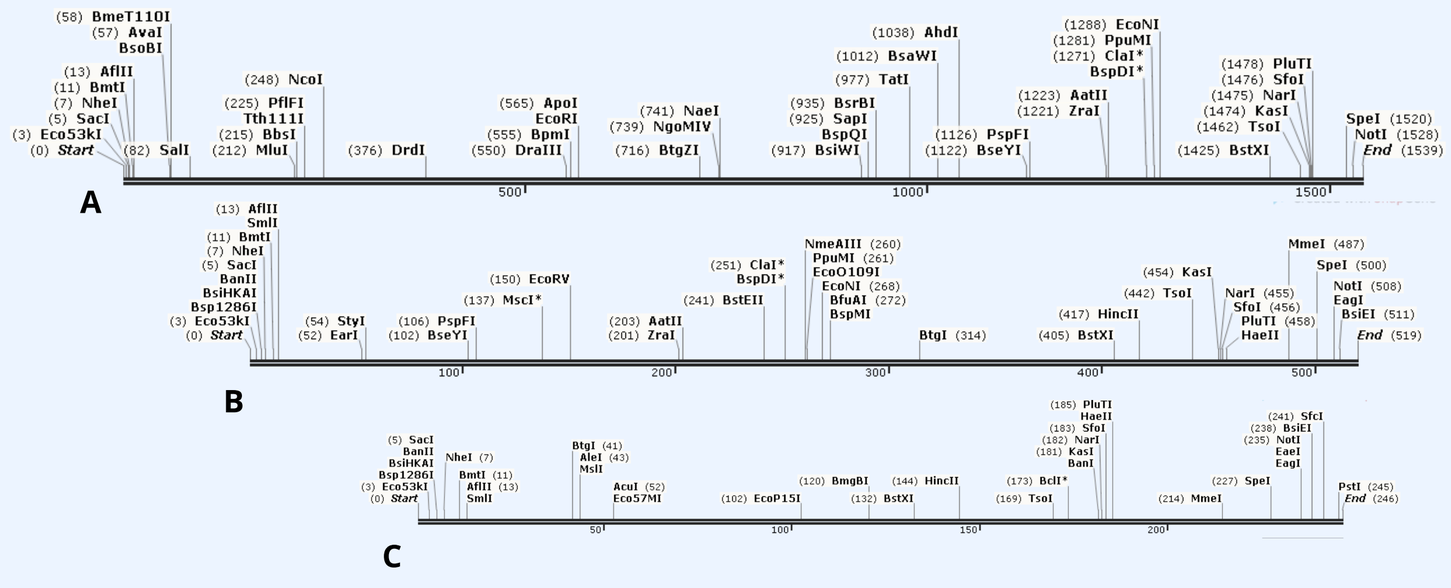

Figure 4. Different variations of lapA C-terminal that will be study phenotipically, where A,B and C are shorter C-terminal modified versions with different sizes.

Future prospects

Phenotypic assays will include:

- Swimming and adhesion assays using different surfaces,
- Biofilm formation curves
- Microscopic assessment of biofilm morphology under different conditions.

We expect that this approach will provide useful insight into the functions of the different domains of LapA and the dynamics of biofilm development in *P. putida*.

References

- [1] Davey, M. E., & O'toole, G. A. (2000). Microbial biofilms: from ecology to molecular genetics. *Microbiology and molecular biology reviews*, *64*(4), 847-867.
- [2] Jahn, A., Griebe, T., & Nielsen, P. H. (1999). Composition of *Pseudomonas putida* biofilms: accumulation of protein in the biofilm matrix. *Biofouling*, *14*(1), 49-57.
- Auerbach, I. D., Sorensen, C., Hansma, H. G., & Holden, P. A. (2000). Physical morphology and surface properties of unsaturated *Pseudomonas putida* biofilms. Journal of bacteriology, 182(13), 3809-3815.
- Martínez-Gil, M., Ramos-González, M. I., & Espinosa-Urgel, M. (2014). Roles of cyclic di-GMP and the Gac system in transcriptional control of the genes coding for the *Pseudomonas putida* adhesins LapA and LapF. Journal of bacteriology, 196(8), 1484-1495.