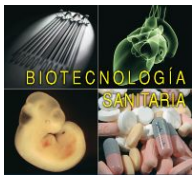
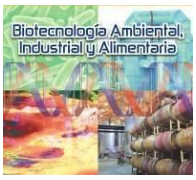
Functional dissection of the large adhesion protein





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ABSTRACT

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. 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. The longest gene in the Gram-negative bacterium *Pseudomonas putida* genome encodes LapA, 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.

The strategy of our project is the construction of different *lapA* variants containing internal deletions of the putative functional domains, and study the role of each of these in LapA-dependent phenotypes. Each version will be inserted in the chromosome of a Δ*lapA* mutant using a Tn*7*-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, and constructs bearing progressively shorter N-terminal domains are underway. Addition of different numbers of repeats will be tested afterwards.

Phenotypic assays will include swimming and adhesion assays using different surfaces, biofilm formation curves and 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*.

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