Poster

Development of a reporter system for screening anti-biofilm activities

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ABSTRACT

Biofilm formation is responsible for increasing antibiotic tolerance in pathogenic bacteria. It is estimated that approximately 80% of chronic infections are associated with this phenomenon. Therefore, the search for therapeutic agents with specific biofilm targets has become of vital importance. One of the main strategies is the search for enzymes that degrade the different components of the extracellular matrix. However, since the composition of the matrix varies among the different microorganisms, an alternative would be to interfere with the signaling cascades that lead to the formation of the biofilm or stimulate its dispersion.

The production of the second intracellular di-cGMP messenger by diguanylate cyclases (DGCs) is a widely conserved process and has a central role in the transition between the planktonic life stage and the biofilm in both Gram positive and Gram negative bacteria, so that high levels promote biofilm formation while low levels induce biofilm dispersal. Molecules with the capacity to inhibit DGC activiy have been identified by screening collections of chemical compounds that are capable of inhibiting biofilm formation, although no metagenomic search has been carried out to date.

The strategy of our project will be the construction of a *P. putida* indicator strain that will helps us to recognize inhibitors of the DGC activity produced by clones from previously constructed environmental meta-libraries. To this end, our strain contains in the chromosome the gene that encodes PleD DGC under the control of the Psal promoter, which is induced by the presence of salicylate thanks to the NahR regulator and a lacZ transcriptional fusion of the PparC promoter of *P. putida*, which is repressed in the presence of di-cGMP.

In the presence of X-gal, a lawn of the indicator strain contained in top agar will be white, while in the case that any of the clones of the meta-library produces a compound capable of inhibiting DGC or interfering with di-cGMP signaling, a blue halo will appear around the colony. To test the operation of the screening system, once the indicator strain was constructed, the ability to form blue haloes was tested in the presence of sulfathiazole, a compound that has demonstrated its ability to inhibit di-cGMP synthesis.

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