MODELING AND DESIGN OF AN ANTIMICROBIAL PEPTIDE FOR HETEROLOGOUS PRODUCTION.

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Introduction. Antimicrobial peptides (AMPs) generate less resistance than antibiotics. They are mostly cationic adopting conformation helix--α at pH7, are amphipathic and interact with membranes penetrating them (Van Loon y col., 2006). Their effectiveness and selectivity are based in the differences between prokaryotes and eukaryotes microorganisms, showing less capacity to break the eukaryotes membranes, due to absence of lipids with negative charge, the lack of a potential gradient of membrane and the presence of cholesterol (Peschel y Sahl 2006). However, the main problem is to obtain an efficient expression system which resists the effect of its own product.

Therefore, the aim of this work is to develop an expression system through the AMP fusion with a complementary peptide that neutralize their charges and activity,resulting in a pro-AMP (no toxic to the producer strain) and so improve performance. The pro-AMP will be purified and subsequently activated through a specific digestion that releases it from its copy.

Methods.

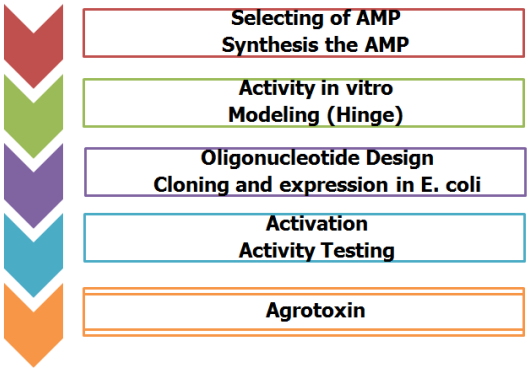

Fig.1 Strategy followed to develop the project.

Results. Expression of the peptide without complementary peptide resulted in the death of the transformants. To neutralize the AMP's charges with a complementary, the structural conformation, degree of confidence and similarities with known proteins, were considered.

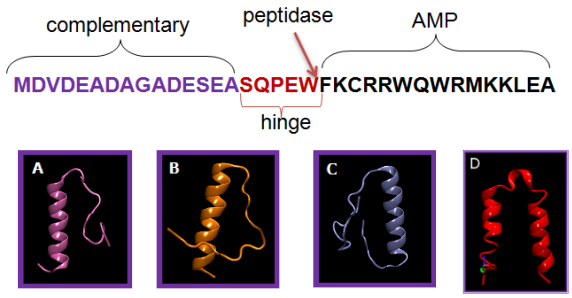

Fig.3 Up. Design of the minigen to express in *E. coli.* **Below** Figure D, choice model for the proposed hinge inactivation with a *C-score* of 1.87.

The choice model was subjected to simulations in box of water and membrane for 100 ns, at 298°K, at 1 atm, 0.1 M of $CaCl_2$ and under periodic condition to the frontier (PCF).

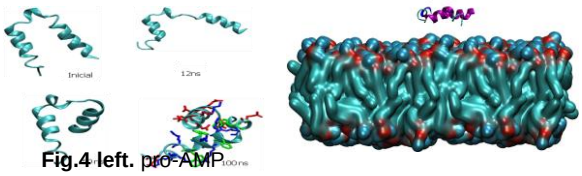

Fig.4 left. pro-AMP simulation in box of water. **Right.** pro-AMP simulation in membrane.

Conclusions. The AMP only be expressed with a complementary peptide in *E. coli,* otherwise it is toxic for the strain.

The choice model showed stability, for that reason was used to obtain the transformant pro-AMP. The next step is purify and release the peptide from its complementary analyzing the activity with bioassays with different phytopathogens.

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