Evaluation of SlyA of a pigmented *Serratia marcescens* isolate

Valeria Medrano[1], Karla L. De Anda[1], Bryant U. Trejo[1], Alexandra M. Montoya[1], Rogelio de J. Treviño[1], Yossef López[2], Angel Andrade[1]

[1]Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Av. Francisco I. Madero s/n, Monterrey, Nuevo León, CP 64460. [2]University of Science and Technology, Thuwal, Saudi Arabia
angel.andradet@uanl.edu.mx

SlyA is a representative member of the ancient family of transcriptional factors (TFs), known as MarR. In *Enterobacteriaceae*, the MarR TFs have been described as global regulators of genes related with stress response, antibiotic resistance, virulence, and catabolism of aromatic compounds. The formation of homodimers is necessary for the DNA interaction of MarR proteins, which recognize palindromic sequences via the 4 recognition helix. Small aromatic carboxylate compounds, such as salicylate, allosterically regulate MarR proteins, reducing their affinity for DNA. Moreover, recent studies suggest that intracellular copper (Cu^{2+}) also affects MarR proteins dimerization, leading to dissociation from DNA.

Despite the central role of SlyA in the regulatory network of *Enterobacteriaceae*, little is known about its contribution in *Serratia marcescens*. A recent report indicates that the elimination of *slyA* in *S. marcescens* negatively impacts prodigiosin production and increases virulence in silkworms. In this study, we aimed to characterize the SlyA protein from a pigmented *S. marcescens* isolate obtained from corneal scrapping. The *slyA* gene was amplified from *S. marcescens* and cloned into pET28 and pBAD33 plasmids. Protein stability at different temperatures was assessed by Western blot after halting protein synthesis in *S. marcescens* using kanamycin. To generate an *slyA S. marcescens* mutant strain, a mutagenic plasmid encoding the upstream and downstream *slyA* DNA sequences was constructed and will be incorporated into *S. marcescens* through homologous double recombination. Additionally, SlyA purification was archived by Ni-NTA chromatography, and its ability to interact with the prodigiosin-associated cluster promoter was evaluated through electrophoretic mobility shift assays. We also demonstrated that SlyA-DNA interaction is affected by the aromatic compounds methyl benzoate and 2′-hydroxy-4′-methoxyacetophenone. *In-silico* molecular docking studies are currently underway to further characterize the interaction between SlyA and these aromatic carboxylate compounds.