

A Short Version of the Amyloid-like Protein TasA Fibrillates and Supports Biofilm Formation in *Bacillus cereus*

J. Caro-Astorga, A. Pérez-García, A. de Vicente, **D. F. Romero**

University of Málaga, Málaga, SPAIN

The formation of bacterial biofilms is a doable thanks to the assembly of an extracellular matrix that provides to the entire community with i) an outstanding architectonic structure and ii) protection to the cells from external aggressions. In *Bacillus subtilis*, a structural element dedicated to the formation of the extracellular matrix is the amyloid-like protein TasA. To form fibers, TasA needs the participation of the protein TapA. Indeed, a *tapA* mutant resembles phenotypically to a *tasA* mutant, which is wrinkle-less pellicles or colonies with no distinguishable morphological features. *tasA* is widely spread within the *Bacillus* genus, but *tapA* is absent in the heterogeneous group of *Bacillus cereus* which includes environmental and pathogenic members; some of them are responsible for important food intoxication outbreaks. Then, we asked whether TasA would still retain functionality in biofilm formation in *B. cereus*. Comparative genomic analysis showed a region in *B. cereus* containing two orthologous of *tasA*, *tasA* and *calY*, and the orthologous of *sipW*, that encodes a signal peptidase. Our mutagenic studies revealed that the entire region was relevant for biofilm formation, and electron microscopy proved the major propensity of TasA than CalY to form fibers in the cell surfaces. These findings also indicated that in *B. cereus* as opposed to *B. subtilis*, an accessory TapA protein is not necessary for the fibrillation of TasA. Indeed, the heterologous expression of this region of *B. cereus* restored the capacity of a *B. subtilis tasA operon* mutant or a single *tasA* mutant to form pellicles. These pellicles stained with the amyloid dye Congo Red and the cells were decorated with fibers, both findings suggestive of an amyloid-like nature of the *B. cereus* TasA. Intriguingly, in a *B. subtilis tapA* mutant, only the entire region of *B. cereus* fully rescued pellicle formation, fibrillation or Congo Red staining, to a lesser extent did *sipW-tasA*, and no restoration was observed with *sipW-calY*. These observations led us to speculate that TapA might cross seed the fibrillation of TasA or CalY in *B. subtilis*. In summary, TasA is relevant for biofilm formation in these two bacterial species, which appears to be governed by its polymerizing nature. The fact that we count with two bacterial species containing versions of TasA with subtle differences will be of great value in our studies of the mechanistic of polymerization of these bacterial amyloid-like fibers and their contribution to the assembly of the extracellular matrix.