

# Structural and polymorphic variability of *Bacillus subtilis* and *Bacillus cereus* TasA amyloid-like biofilm filaments

SpT.02-12

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*Bacillus subtilis* forms an extracellular biofilm matrix that is mainly composed of the protein TasA and an exopolysaccharide. Previous studies indicate that TasA forms amyloid-like filaments within the biofilm matrix, although the characteristic amyloid X-ray diffraction pattern has never been observed to confirm the presence of a cross-beta structure in TasA filaments. Here, we report the combination of solution NMR, solid-state NMR and X-ray diffraction to investigate the protein conformation in TasA filaments. TasA monomers are unstructured in solution, and X-ray diffraction analysis reveals a typical cross-beta stacking in the filamentous state, with additional reflections not characteristic for a cross-beta arrangement. Solid-state NMR line-widths indicate a high structural order of TasA molecules in the filaments, but intriguingly, chemical shift-based analysis shows that the TasA conformation in the filamentous state contains both beta-sheet and  $\alpha$ -helical secondary structure elements. A comparison of solid-state NMR signatures of both *Bacillus subtilis* and *Bacillus cereus* TasA filaments reveals an only partially conserved fold of TasA with distinct  $\alpha$ -helical propensity in the two species. Significant differences are observed for the structural polymorphism in *B. subtilis* and *B. cereus* filaments through solid-state NMR line broadening, suggesting a structural and dynamic variability during the TasA filament assembly process between different bacterial species.