**Session:** Session 5: Synthesis and Assembly and Function of the Biofilm Matrix  
**Date and Time:** Tuesday, October 9, 2018, 10:50 am - 12:45 pm  
**Abstract Title:** Agd3 is a Novel Polysaccharide Deacetylase Integral to *Aspergillus fumigatus* biofilm formation  
**Author Block:**  
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*Aspergillus fumigatus* is an opportunistic fungal pathogen that causes chronic and acute invasive infections. The exopolysaccharide galactosaminogalactan (GAG) is a virulence factor essential for *A. fumigatus* biofilm formation. GAG aids in host immune evasion, adherence to tissue and surfaces, as well as antifungal resistance. After GAG polymerization and export across the cell membrane, the N-acetylgalactosamine component of the polymer is partially deacetylated by the secreted protein Agd3. Although, the homology to characterized members is low, bioinformatics analysis found Agd3 had similarity to the carbohydrate esterase 4 (CE4) family. We have previously shown that Agd3 deacetylation of GAG renders the polymer adherent to multiple surfaces including the fungal cell wall and mediates virulence. Thus, Agd3 represents an attractive target for development of novel therapeutics. We have recombinantly expressed Agd3 in *Pichia pastoris* and determined its structure to 2.8 Å resolution using the single-wavelength anomalous dispersion technique. The structure revealed a compact three-domain architecture not previously observed in CE4 enzymes. The deacetylase domain has an active site groove that is elongated by the smaller N-terminal domain, thus creating an extended cleft on one face of the enzyme. Recombinant Agd3 is active on GAG and when added exogenously can rescue biofilm formation of Δagd3. Enzyme-linked lectin assays (ELLAs) and MALDI-TOF mass spectrometry of Agd3 reaction products show that Agd3 requires at least octasaccharides for efficient activity and point mutants of the putative catalytic residues abolish this activity. The N-terminal domain (Agd3¹⁴¹⁻³⁶⁵) was produced in isolation and in ELLA assays showed dose dependent binding of secreted GAG. *Aspergillus* fluorescence staining using labeled Agd3¹⁴¹⁻³⁶⁵ demonstrates co-localization with the GAG-specific lectin soybean-agglutinin. Our results show that Agd3 is a GAG deacetylase that represents a new family of CE enzymes which spans fungal and bacterial kingdoms. The N-terminal domain of Agd3 is the first identified and characterized member of a new CBM family. Combined, the structure and our functional characterization of the protein shed light on the mechanism of GAG maturation and enriches our understanding carbohydrate active enzymes.