CONSIDER FOR TALK

9th Annual SEA-PHAGES Symposium Abstract

Virginia Commonwealth University

Richmond VA

Corresponding Faculty Member: Allison Johnson (aajohnson@vcu.edu)

Biochemical and structural characterization of Bacillus phage Endolysins

Lucas Rizkalla, Louise Temple\*, Dan Nelson, Phil Mosier, Allison Johnsons

\* James Madison University, Harrisonburg VA

Our story begins with a ‘bioinformatics discovery’ of a SEA PHAGES undergraduate student in the in silico lab course. Phage endolysins are comprised of two domains, an N-terminal catalytic domain and a C-terminal cell-wall binding domain. This student carefully characterized the sequence diversity and domain structure of a collection of Bacillus phage endolysins. We were able to identify a collaborator with endolysin expertise and move that work forward to structurally and biochemically characterize of three of these endolysins. Endolysin proteins from Bacillus phages Anthos (A54), Nigalana (N74) and TsarBomba (TB40) were expressed, purified and characterized for endolysin activity. These three proteins possess the same cell wall binding domain, but have three different N-terminal catalytic domains. Dose response curves, temperature stability, pH profiles and salt dependence were determined for all three proteins for lysis against B. cereus 4342. The main difference between the three enzymes is that Anthos A54 endolysin appears ~3-fold more active than the other two enzymes. Host range testing shows these three endolysin proteins behave with a similar host profile, and were able to lyse a variety of Bacillus species. Unfortunately, they do not lyse B. anthracis. Finally, the catalytic domains of these proteins were computationally modeled in comparison with their closest structural homologs to illustrate conservation of overall all fold as well as active site amino acids required for metal binding and peptidoglycan cleavage. This study revealed biochemical and host range similarity despite sequence and structural differences, suggesting Bacillus phages use a conserved mechanism to lyse Bacillus species.