Department of Biological Sciences
Ph.D. Viva Voce

Speaker: **Mr. Himanshu Joshi** Advisor: **Dr. Vikas Jain**
Date and Time: **Monday, April 30, 2018 at 10:00 AM** Venue: **AB 3, #401**

Molecular insights into the function of lysozyme domain of mycobacteriophage D29 peptidoglycan hydrolase

Abstract

Mycobacterium tuberculosis (Mtb) has posed a grave threat to the global healthcare for decades. Although antibiotics have helped to curtail Mtb related fatalities, the emergence of drug resistant bacteria has warranted the search for newer potent drugs and therapeutics. Mycobacteriophages in this regard present a unique avenue for the development of new generation therapeutics. During the mycobacteriophage life cycle, concerted and timely action of three phage-encoded proteins *viz*. Holin, Lysin A, and Lysin B lead to the disruption of cytoplasmic membrane, cell wall, and outer mycolic acid layer of mycobacteria, respectively. This ultimately leads to host cell rupture.

In this study, we present an in-depth analysis of the structure-function relationship of the lysozyme domain (LD) of the *Mycobacterium* phage D29-encoded endolysin, Lysin A. LD demonstrates peptidoglycan hydrolase activity against both Gram-positive *Micrococcus lysodeikticus* and Gram-negative *E. coli*. We adopted a novel strategy to identify key residues involved in the activity of LD. Replacement of these residues with Ala or other amino acids results in loss of activity and/or stability. Interestingly, these residues were also found to be evolutionarily conserved. Thus we were able to successfully map the active site of the enzyme by *in vitro*, *in vivo*, and *in silico* experiments and show how mutations affect the enzyme active site. Additionally, using LD protein, we have developed a novel method to expedite the protein purification process from both high-throughput as well as large-scale culture. The protein of interest that is co-expressed with the LD can be easily isolated and purified from a large-scale culture.

We thus present LD as a potent candidate for developing phage-based broad spectrum therapeutics as well as a novel tool in recombinant DNA technology application.

References

1. Pohane, A. A., Joshi, H., and Jain, V. (2014) *J Biol Chem*, **289**, 12085-12095
2. Joshi, H., Seniya, S. P., Suryanarayanan, V., Patidar, N. D., Singh, S. K., and Jain, V. (2017) *FEBS Lett*, **591**, 3276-3287
3. Joshi, H., and Jain, V. (2017) *Anal Biochem*, **528**, 1-6