INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	**SURBHI KUMARI**
Roll Number	:	**206106022**
Programme of Study	:	**Ph.D.**

Thesis Title : **Study on Caseinolytic protease chaperones and its cognate adaptors of *Leptospira interrogans***

Name of Thesis Supervisor(s)	:	**Prof. Manish Kumar**
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SHORT ABSTRACT

Bacterial Caseinolytic protease (Clp) systems are central to protein quality control, mediating the ATP-dependent degradation of misfolded or aggregated proteins. In *Leptospira interrogans*, the Clp machinery comprises two proteolytic isoforms (ClpP1 and ClpP2), multiple ATPase chaperones (ClpA, ClpC, ClpX), and adaptor proteins (ClpS1, ClpS2). The core protease forms a hetero-tetradecameric ClpP1P2 complex that exhibits intrinsic peptidase activity toward short peptides, while degradation of protein substrates requires association with ATPase chaperones such as ClpX. However, the structural basis for selective activation and regulation of leptospiral Clp components remains poorly understood.

This study investigates key structural motifs of ClpP isoforms and the functional interplay of chaperones and adaptor proteins in controlling protease activity. Five site-specific mutants targeting conserved regions of ClpP1 and ClpP2 were analyzed. Two ClpP2 variants (S40AK41N and Y62A) displayed enhanced peptidase activity independent of ClpP1 and exhibited increased hydrodynamic size, suggesting altered conformations. Their corresponding heterocomplexes showed elevated activation by the natural antibiotic ADEP1 (1.5–1.7-fold increase) and a threefold rise in recruitment of the physiological substrate trigger factor (LinTF). Conversely, mutations in ClpP1 (E170D, N172D) reduced activity by ~50%, and deletion of the IG motif in ClpP2 abolished function, identifying critical residues required for proper activation of the ClpP1P2 complex.

Functional characterization of chaperone components revealed that LinClpC undergoes nucleotide-driven oligomerization and associates with both ClpP isoforms with similar affinity.

LinClpC–ClpP1P2 complexes degrade casein even without ATP, with ATP/ATPγS further enhancing activity, whereas ADEP1 acts as a more potent activator than ClpC. Structural and biochemical analyses of regulatory elements demonstrated that LinClpA, composed of an N-terminal domain and two AAA+ ATPase modules, forms nucleotide-dependent oligomers whose activity is stimulated by ClpP1 and ClpP2 but inhibited by adaptors ClpS1 and ClpS2. A truncated variant lacking the N-terminal domain (LinClpA$^{\Delta N}$) retains oligomerization and shows increased ATPase activity, but is unresponsive to adaptor-mediated regulation, underscoring the importance of the N-terminal domain in substrate-selection pathways. Additionally, the SsrA C-degron tag was shown to facilitate substrate delivery to the leptospiral ClpAP1P2 machinery.

Collectively, these findings provide new mechanistic insight into the structural determinants and regulatory interactions that govern Clp protease function in *L. interrogans*. This work advances our understanding of bacterial proteostasis and highlights several components of the leptospiral Clp system as potential targets for antimicrobial intervention.