INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	**Md. Saddam Hussain**
Roll Number	:	**166106005**
Programme of Study	:	**Ph.D. in Biosciences and Bioengineering**

Thesis Title: **Functional elucidation of CRISPR-Cas I-B interference machinery of *Leptospira interrogans***

Name of Thesis Supervisor(s)	:	**Prof. Manish Kumar**
Thesis Submitted to the Department/ Center	:	**Department of Biosciences and Bioengineering**
Date of completion of Thesis Viva-Voce Exam	:	**21st August 2023**
Key words for description of Thesis Work	:	***Leptospira interrogans,* Leptospirosis, CRISPR-Cas type I-B, Cascade, LinCas7, Cas7, LinCas8b, Cas8, LinCas11b, Cas11, PAM, DNase, RNase**

SHORT ABSTRACT

Leptospira interrogans is the causative agent of leptospirosis, a zoonotic disease accounting for approximately 60,000 human deaths every year globally. The leptospires show incompetence to conventional genetic manipulation tools, and therefore, the molecular mechanism of its pathogenesis remains poorly comprehended using the reverse genetics approach. One possible reason for its incompetence in genetic manipulation is the presence of the CRISPR-Cas system in its genome. A CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated proteins) is an RNA-directed inheritable adaptive immunity in prokaryotes against invasive mobile genetic elements (MGEs), including bacteriophages and plasmids. The system encodes an effector complex (Cascade) that utilizes small crRNA (CRISPR RNA) to sense and interfere with the invasive MGEs having the crRNA-complementary sequence next to a protospacer adjacent motif (PAM). The predominant CRISPR-Cas type I-B system, marked in several pathogenic *Leptospira* genomes, presents a promising alternative to be explored as an endogenous genome editing tool. Moreover, to exploit *Leptospira's* CRISPR-Cas type I-B (Lin_I-B) system for targeted genetic manipulation, characterization of its interference machinery and associated PAMs is a prerequisite. To this end, the present study reports the molecular and functional characterization of interference machinery of a Lin_I-B system, recently identified in *L. interrogans* serovar Copenhageni strain Fiocruz L1-130. The comprehensive biochemical analysis of the LinCas7, a major subunit of the Cascade, revealed it to be a Mg^{2+} ion-dependent non-specific endoDNase, unlike other Cas7 family proteins. Moreover, LinCas7 exhibits distinct binding to crRNA in the presence of Mg^{2+} ions, testifying its canonical role in Lin_I-B defense response. The molecular characterization of LinCas8b disclosed it as a genetic fusion of large (LinCas8b) and small subunit (LinCas11b) proteins of the Cascade (LinCascade). In this study, LinCas11b is demonstrated to co-translate from an in-frame internal translation start codon encoded within the *lincas8b* gene. LinCas11b displays structural and functional analogy with the well-studied Cas11 family proteins. In addition, the interference machinery of the Lin_I-B, when expressed in a surrogate host *E. coli,* was able to annihilate the target DNA with the predicted PAM. In sum, the current study presents the molecular and functional insight of the *Leptospira* subtype I-B interference machinery that, soon, may pave the way for scientists to harness the system as a programmable endogenous tool for genetic manipulation.