CHAPTER VII

Conclusion and Future Perspectives

7.1 Conclusion

The study highlights the significance of structure-function relationships in fibrinolytic enzymes from the *Bacillus* genus, emphasizing their catalytic mechanisms, stability, and adaptability under various environmental conditions. Through in-silico analyses, the conserved catalytic triad (Asp, His, Ser) and physicochemical properties like thermostability, alkaline stability, and solubility were shown to contribute to their industrial and pharmaceutical potential. Structural models and domain analyses validate these enzymes' stability and functional capabilities. Notably, enzyme sequences of *Bacillus amyloliquefaciens* and *Bacillus subtilis* exhibit promising properties such as high thermostability, αβ-fibrinogenase activity, and efficient tissue penetration due to their mid-molecular weights and solubility. These findings provide a strong theoretical framework for designing mutant enzymes with enhanced properties for therapeutic and industrial applications, underscoring the potential of *Bacillus*-derived fibrinolytic enzymes in biotechnology and drug development.

The potential of microbial sources, specifically *the Bacillus megaterium* N1 strain, was highlighted as bio-factories for fibrinolytic enzyme production. The N1 strain, isolated from the alcohol-producing starter culture, demonstrated significant fibrinolytic activity by degrading all three fibrin polypeptide chains within 90 minutes of incubation. Molecular characterization confirmed its identity as *Bacillus megaterium*, reinforcing its utility in industrial and therapeutic applications. Unlike traditional fibrinolytic drugs, the direct-acting fibrinolytic protease from the N1 strain does not require plasminogen activators, reducing the risk of side effects such as platelet activation. This finding underscores the promise of N1-derived enzymes in managing thromboembolic disorders while paving the way for further exploration of its biochemical properties, protein engineering potential, and pre-clinical safety for therapeutic use.

A recombinant fibrinolytic protease from *B. subtilis* was successfully developed, and its sequence was optimized for expression in an *E. coli* system. It demonstrated its potential as a therapeutic agent for thrombotic diseases. The recombinant enzyme, purified through affinity chromatography, exhibited moderate fibrinolytic activity by preferentially degrading fibrin's α-chain and demonstrated thrombolytic and anticoagulant activities. In vivo studies confirmed its efficacy in prolonging clotting time and its safety, as no hemolysis, toxicity, or adverse effects were observed. While the recombinant protease showed slightly lower anticoagulant

potency than nattokinase, its targeted action and safety profile highlights its promise as a novel therapeutic prototype for managing cardiovascular conditions.

7.2 Future perspectives

This study's findings highlight the necessity for extensive research on the pharmacokinetics and pharmacodynamics of the recombinant protease. Such investigations are crucial for establishing the protease's optimal dose, effectiveness, and safety profiles across many biological systems. Furthermore, examining its mechanism of action at both molecular and systemic levels will yield essential insights into its therapeutic potential and constraints.

Additional research is advised to improve the bioavailability of the recombinant protease. Creating thrombus-targeting medication delivery devices may enhance localization at the location of clots. This method may improve the enzyme's efficacy while minimizing off-target effects and systemic toxicity. These advancements are anticipated to facilitate the development of effective cardiovascular medications with improved therapeutic results, ultimately allowing the recombinant protease to advance to clinical trials with increased confidence in its potential as a safe and efficacious treatment for thrombotic disease.

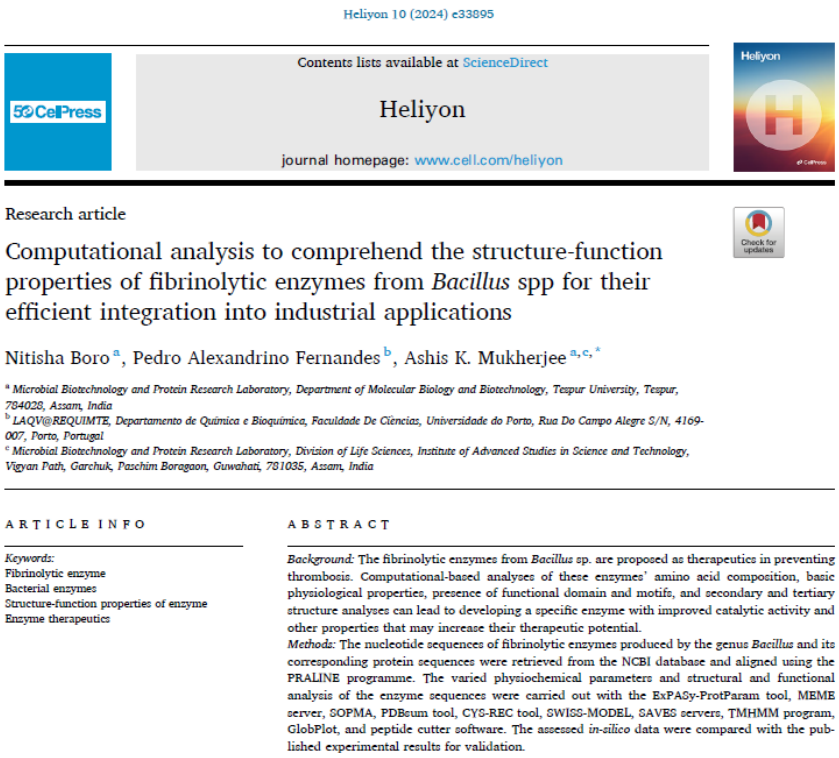
PUBLICATIONS AND CONFERENCES

A. Publications in peer-reviewed international journals from thesis work

1. **Boro, N.,** Fernandes, P. A., and Mukherjee, A. K. (2024). Computational Analysis to Comprehend the Structure-Function Properties of Fibrinolytic Enzymes from *Bacillus* spp for their Efficient Integration into Industrial Applications. **Heliyon**, 10(13). **IF-3.4**
2. **Boro, N.,** Roy, A., and Mukherjee, A. K. (2024). Biochemical and In vivo Pharmacological Characterization of a bacterial non-toxic fibrinolytic serine protease showing thrombolytic activity ***(Manuscript communicated).***

B. Presented at National and International conferences

1. **Boro, N.,** Fernandes, P.A., and Mukherjee, A. K. (2022) "Characterization and understanding of the structure-function properties of *Bacillus* spp. Fibrinolytic enzymes by computational analysis" at the **14th Annual Meeting of the Proteomics Society, India and International Conference on Proteins & Proteomics (PSI-ICPP 2022)**, CSIR-Indian Institute of Chemical Biology, Kolkata, 03-05, November, 2022.



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Research article

Computational analysis to comprehend the structure-function properties of fibrinolytic enzymes from *Bacillus* spp for their efficient integration into industrial applications

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ABSTRACT

Background: The fibrinolytic enzymes from *Bacillus* sp. are proposed as therapeutics in preventing thrombosis. Computational-based analyses of these enzymes' amino acid composition, basic physiological properties, presence of functional domain and motifs, and secondary and tertiary structure analyses can lead to developing a specific enzyme with improved catalytic activity and other properties that may increase their therapeutic potential.
Methods: The nucleotide sequences of fibrinolytic enzymes produced by the genus *Bacillus* and its corresponding protein sequences were retrieved from the NCBI database and aligned using the PRALINE programme. The varied physiochemical parameters and structural and functional analysis of the enzyme sequences were carried out with the ExPASy-ProtParam tool, MEME server, SOPMA, PDBsum tool, CYS-REC tool, SWISS-MODEL, SAVES servers, TMHMM program, GlobPlot, and peptide cutter software. The assessed *in-silico* data were compared with the published experimental results for validation.