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Notice for the PhD Viva Voce Examination

Ms Sompalli Bhavana (Registration Number: 1940090), PhD scholar at the School of Sciences, CHRIST (Deemed to be University), Bangalore will defend her PhD thesis at the public viva-voce examination on Friday, 25 October 2024 at 11.00 am in Room No. 044, Ground Floor, R & D Block, CHRIST (Deemed to be University), Bengaluru - 560029.

Title of the Thesis	:	**Production and Purification of Fibrinolytic Enzymes from Mangrove Sediment Microbiota and Study of its Clot Lysis Activity**
Discipline	:	**Biotechnology**
External Examiner - I	:	**Dr Tartte Vijaya** Professor Department of Botany Sri Venkateswara University Tirupati – 517502 Andhra Pradesh
External Examiner - II	:	**Dr Vedamurthy A B** Professor P G Department of Studies and Research in Biotechnology and Microbiology Department of Applied Genetics Karnatak University Dharwad, Karnataka
Supervisor	:	**Dr Alok Kumar Malaviya** Associate Professor Department of Life Sciences School of Sciences CHRIST (Deemed to be University) Bengaluru – 560029 Karnataka

The members of the Research Advisory Committee of the Scholar, the faculty members of the Department and the School, interested experts and research scholars of all the branches of research are cordially invited to attend this open viva-voce examination.

Registrar

Place: Bengaluru
Date: 18 October 2024

ABSTRACT

Cardiovascular diseases are the leading cause of mortality in the present generation, and the WHO has stated globally that 17 million people are being affected by heart attacks. Nowadays, cardiovascular (CVD) disorders are treated using fibrinolytic enzymes. The development of new cost-effective procedures for the low-cost manufacture of these life-saving enzymes is necessary since the existing fibrinolytic enzymes are quite expensive and have several undesirable side effects. We began our work in this approach with the screening and isolation of fibrinolytic enzyme producers, since the availability of an appropriate strain is thought to be the initial step for any process development endeavour. The objective of this study was to identify and evaluate fibrinolytic enzyme-producing microbiota from the largely unexplored mangrove habitat. 7 of the 200-protease enzymes-producing isolates were found to show fibrinolytic activity after screening 30 samples of mangrove soils. Out of these seven, the bacterial strain (AIBL_AMSB2) that produced the highest level of fibrinolytic enzyme activity was characterized using biochemical and genomic techniques before being identified as *Bacillus subtilis* subsp. *inaquosorum* by 16S rRNA analysis. The strain was also observed to produce the enzyme amylase. Using random mutagenesis methods (UV and EMS), *Bacillus subtilis* AIBL_AMSB2 was improved to *Bacillus subtilis* AIBL_AMSB2_M7E32, resulting in a 54.70 % increase in fibrinolytic activity.

Media components involving carbon and nitrogen sources, metal ions, incubation temperature, and pH that can impact fibrinolytic enzyme production were optimized by the OFAT method using the improved strain. Additionally, response surface methodology was used to further enhance fibrinolytic enzyme production. The findings showed that yeast extract and MgSO4 were the crucial media components having a positive impact on the enzyme activity, and we could achieve a 200 % increase in the enzyme activity after media optimization. Batch fermentation with unoptimized and optimized skim milk media was carried out in a 5 lit in- situ fermenter that resulted in a 208 % increase in protease activity, and a 30 % rise in fibrinolytic activity using optimized media when compared to the unoptimized media. A 4-step purification process was used to partially purify the enzyme, where a purification fold of 43.33 with a specific activity of 6368.20 (U/mg) was achieved. The purified enzyme molecular weight was determined by SDS PAGE as a 28 kDa protein and its fibrinolytic activity was further confirmed on a zymogram. The purified enzyme's *in vitro* activity on blood clots showed a 72.96 % clot lysis after 48 hours of incubation, confirming its thrombolytic activity.

Keywords: Mangroves, Cardiovascular diseases, Fibrinolytic enzyme, Strain improvement, Fermentation, Response Surface Methodology (RSM), Purification, Invitro activity

Publications:

1. Sompalli B, Malaviya A. A novel fibrinolytic enzyme producer from mangrove soil sediments: Screening, isolation, strain improvement, and fermentation. J App Biol Biotech. 2024;12(2):182-190. http://doi.org/10.7324/JABB.2024.161429
2. Sompalli B, Malaviya A. Purification and evaluating *in vitro* activity of a fibrinolytic protease produced by a mangrove isolate *Bacillus subtilis* AIBL_AMSB2_M7E32. J App Biol Biotech. 2024;12(5):237-242. http://doi.org/10.7324/JABB.2024.175220