(122)

DEPARTMENT OF BIOTECHNOLOGY

MINISTRY OF SCIENCE AND TECHNOLOGY, GOVT. OF INDIA

FUNDED RESEARCH PROJECT

Pharmacological studies of recombinant and mutant fibrin(ogen)olytic protease(s) for the prevention and/or treatment of hyperfibrinogenemia associated cardiovascular disorders

FIRST ANNUAL REPORT

(7th September, 2018 to 6th September, 2019)

Submitted By

Prof. Ashis Kumar Mukherjee
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Department of Molecular Biology and Biotechnology,
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Assam, India

Prof. Uttam Chanda Banerjee
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Mohali, Chandigarh-160062
Punjab, India

DEPARTMENT OF BIOTECHNOLOGY, INDIA

1	**Duration of the project**	07th September, 2018 to 06th September, 2019
2	**Project Title**	Pharmacological studies of recombinant and mutant fibrin(ogen)olytic protease(s) for the prevention and/or treatment of hyperfibrinogenemia associated cardiovascular disorders
3	**Principle Investigators:**	Dr. Ashis Kumar Mukherjee, Tezpur University, Tezpur-784028 Dr. Uttam Chanda Banerjee, NIPER, Mohali, Chandigarh-160062
4	**Objectives sanctioned**	**1.** Cloning and expression of purified recombinant fibri(ogeno)lytic enzyme **2.** Biochemical and pharmacological characterization of purified recombinant protease, and comparison with commercial anticoagulant/fibrinogenolytic drugs **3.** Improvement of fibrinogen binding potency of recombinant fibrinogenolytic serine protease for enhancing its catalytic activity
5	**Objectives achieved**	Cloning and expression of recombinant fibrin(ogeno)lytic protease catalytic activity of recombinant protease
6	**Human resource development** **i. JRF/SRF**	JRF appointed in this project
7	**Research outcome** **Publication (list may be included as annexure)**	No
	Patents	No
	Technology developed/ commercialized	No
8	**Summary of progress work:**	Fibrinogenolytic enzyme producing Gram positive *Bacillus sp.* was isolated from fermented food sample of NE India. The primers to amplify the fibrinogenolytic gene from this bacterium were designed and the fibrinogenolytic encoding gene sequence was PCR amplified. The gene sequence was confirmed by DNA sequencing. This was followed by synthesis of protease gene by codon

		optimization. The synthetic gene containing pro-peptide and mature enzyme sequence was cloned in between the NcoI and XhoI site of pET26b(+) expression vector containing pelb leader sequence and His-tag protein. The insertion of sequence in the correct orientation was ascertained by double digestion of recombinant expression vector. The recombinant pET26b(+) vector was transformed into BL21a competent cells and transformed cells were grown in LB agar medium containing kanamycin. Few of the selected colonies appeared post 16 h of growth were allowed to grow in LB broth at 37°C until the cell density reached 0.4-0.5 at 620 nm. Protein expression was induced with 1 mM IPTG followed by growing the cells for another 24 hr at 37°C. The recombinant protein expression in inclusion bodies was confirmed by SDS-PAGE and Western blot analyses by anti-his tag antibody. The recombinant protein containing his-tag in its c-terminal was isolated by Ni-NTA affinity column chromatography. The isolated recombinant protein showed fibrinogenolytic activity by SDS-PAGE analysis; however, the activity was found to be transient and warrants to find out the mechanism to stabilize the enzyme.The catalytic activity of the recombinant fibrinogenolytic protease was carried out by growing at 37°c and plotting the growth curve of wet cell mass vs. absorbance. Glucose and malt extract have been found to be optimum carbon and nitrogen sources, respectively. Further optimization of parameters affecting the fermentation conditions is being carried out by one factor at a time as well as by statistical approach.

9. Brief progress report:

Flowchart depicting the cloning and expression of bacterial fibrinogenolytic protease gene

Screening of fibrinogenolytic enzyme producing bacteria on fibrin agar plates by serial dilution

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Chromosomal DNA isolation and PCR amplification of fibrinogenolytic encoding gene

↓

Sequencing of PCR amplified fibrinogenolytic gene sequence

↓

Codon optimization and synthesis of protease gene by ligating with pET-26b(+) vector between NcoI and XhoI sites

↓

Preparation of BL21a competent cells by Calcium chloride method

↓

Transformation of synthetic protease gene into BL21a competent cell by heat shock method

↓

Plasmid isolation from recombinant clone and RE digestion with NcoI and XhoI enzymes

↓

Inoculation of recombinant cell into LB broth

↓

Protein expression induced by 1.0 mM IPTG after cell density reached ~0.6 OD at 620 nm

↓

Isolation of expressed protein by dialysis

↓

12.5% SDS-PAGE and western blot by His-tag Ab for expressed protein

↓

Purification of expressed protein by Ni-NTA affinity column

↓

Fibrinogenolytic assay of eluted protein by 12.5% SDS-PAGE

↓

2% inoculum incubated in 50mL media at 37°c for 15 h

↓

Plotting of growth curve of wet cell mass vs. absorbance

↓

reducing sugar content in the broth was measured by the DNS method

INSTITUTE WISE OBJECTIVES AND PROGRESS OF WORK:

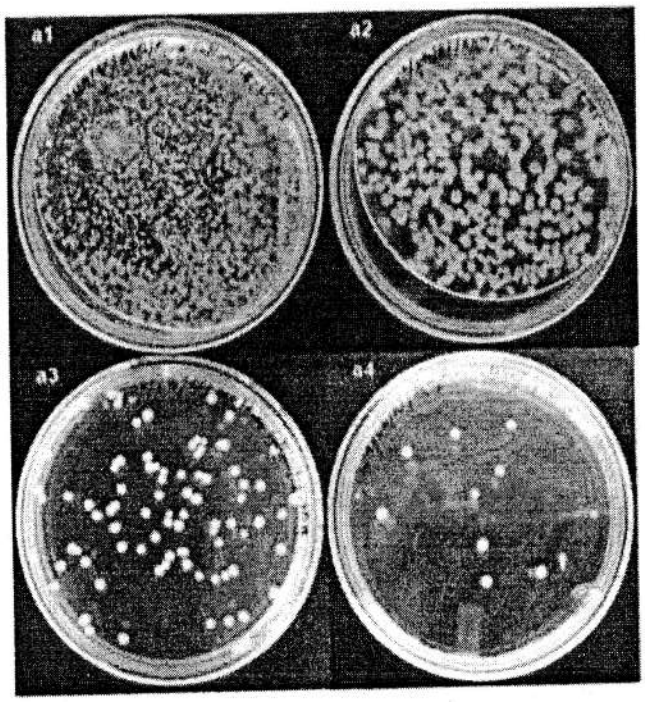
I. Tezpur University :

1. Cloning and expression of purified recombinant fibri(ogeno)lytic enzyme:

1A. Screening, DNA isolation and PCR amplification of fibrinogenolytic gene

1 gm of fermented food sample was mixed with 9.0 ml of 0.9% (w/v) sterile saline and serially diluted up to 10^{-4} dilution. Then 100 µl aliquot from the 10^{-4} diluted fermented food sample was spreaded on fibrin-agar plate containing fluconazole (50 mg/ml). After 24 h incubation at 37°C visible colonies (5 no.) from fibrin plates were inoculated in 10 ml of LB broth and allowed to grow until cell density reached ~0.5 OD at 600 nm. The broth culture was serially diluted upto 10^{-4} dilution as proceeded above for obtaining pure colonies and plated on fibrin plates (Fig.1.a). A colony from 10^{-4} dilution fibrin plate was selected for the isolation of genomic DNA (which was identified as *Bacillus sp.*) by GeneJET genomic DNA purification kit. ~200 ng of isolated DNA was being used for PCR amplification of fibrinogenolytic encoding gene with a set of forward primer F_1 5′-AGACCATGGGATGGCAGGGAAATC -3′ (NcoI site underlined) and reverse primer R_1 5′-AGACTCGAGCTGAGCTGCCGCCTG -3′ (XhoI site underlined) with the PCR condition as: 94°C, 5 min, followed by 30 cycles consisting of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 2 min. The amplified PCR product size was found to be ~1 kb (Fig. 1b) and the protease gene was confirmed by sequencing (Fig.1.c).

a



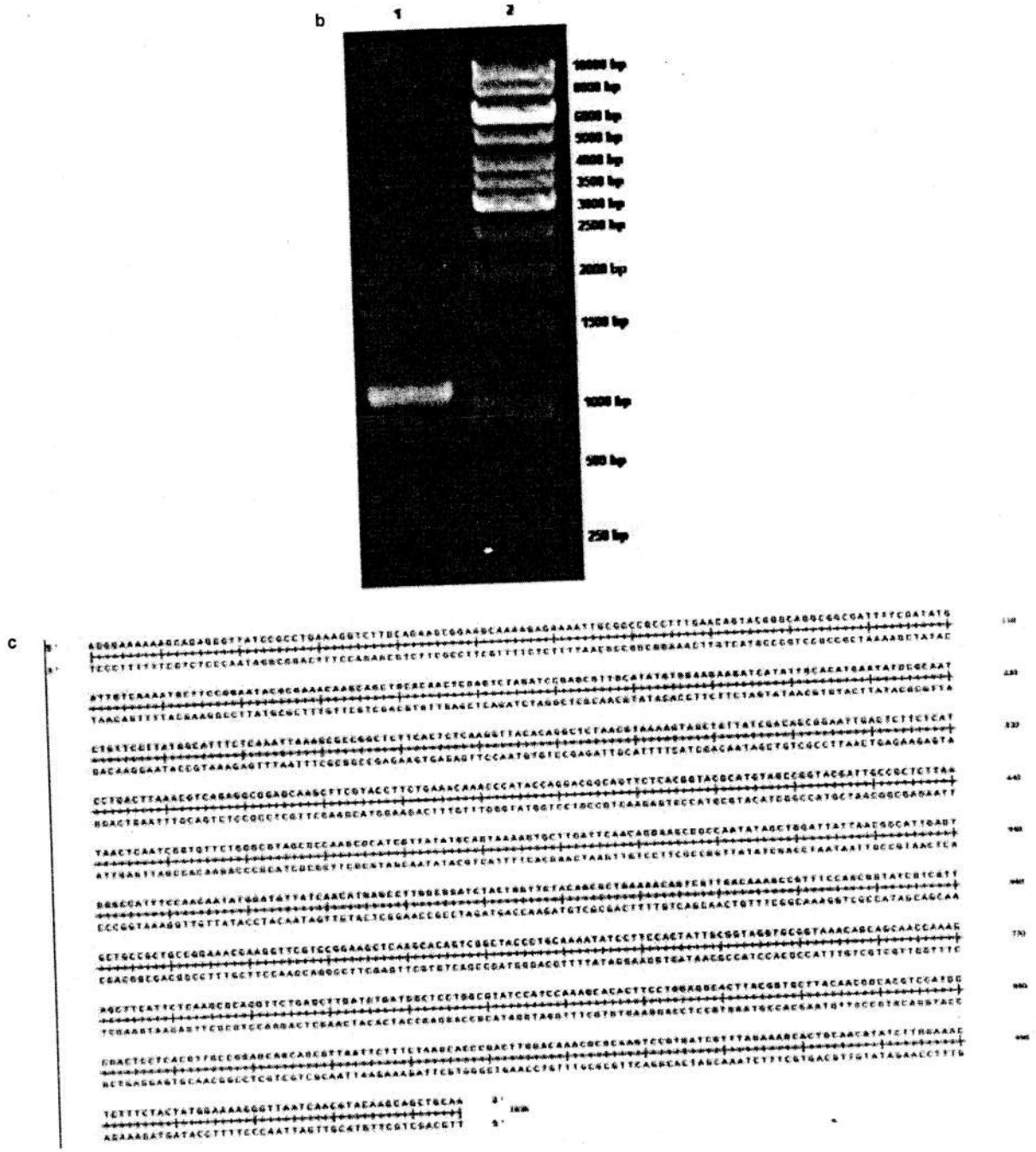


Fig.1A. a. Screening of fibrinogenolytic producing bacteria on fibrin plate by serial dilution (a1-a4) 10^{-1}, 10^{-2} 10^{-3} and 10^{-4} respectively. b. PCR amplification of fibrinogenolytic encoding gene; Lane1, PCR product; Lane2, DNA marker. c. Sequencing result

1B. Synthesis of protease gene, transformation and plasmid isolation

The PCR amplified fibrinogenolytic gene sequence was codon optimized for the synthesis of protease gene by *E. coli* B 21 cells (Fig.1B-a). The optimized gene sequence (1073 bp) containing the enzyme prosequence was inserted downstream of the pelB signal sequence in between the NcoI and XhoI restriction sites of pET-26b(+) expression vector and also had an extra six His codons at the 3' end. About 200 ng of recombinant pET-26b(+) vector was transformed into BL21 competent cells by heat shock method and cells were evenly spreaded on pre-made LB agar plates containing Kanamycin (50 µg/ml). Appearance of 15-20 colonies were observed after 16 h incubation at 37°C. Three colonies were selected for the isolation of recombinant plasmid and double digestion with NcoI and XhoI enzymes to ensure successful transformation (Fig.1B-b).

a

GCGGGCAAAAGCAACGGCGAGAAGAAGTACATCGTGGGTTTCAAGCAGACCATGAGCACCATGAGCGCGGCGAAG
AAGAAGGACGTGATCAGCGAGAAGGGTGGCAAAGTGCAGAAGCAATTCAAATACGTTGACGCGGCGAGCGCGACC
CTGAACGAGAAGGCGGTGAAAGAACTGAAGAAAGACCCGAGCGTTGCGTATGTGGAGGAAGATCACGTTGCGCAG
GCGTACGCGCAAAGCGTGCCGTATGGCGTTAGCCAGATTAAGGCGCCGGCGCTGCACAGCCAAGGTTTCACCGGC
AGCAACGTGAAAGTTGCGGTGATCGACAGCGGTATTGATAGCAGCCATCCGGACCTGAAAGTGGCGGGTGGCGCG
AGCATGGTTCCGAGCGAAACCAACCCGTTTCAGGATAACAACAGCCACGGCACCCATGTGGCGGGCACCGTGGTT
GCGCTGAACAACAGCGTTGGTGTGCTGGGCGTTGCGCCGAGCGCGAGCCTGTACGCGGTTAAAGTGCTGGGCGCG
GACGGTAGCGGCCAATATAGCTGGATCATTAACGGTATCGAATGGGCGATTGCGAACAACATGGACGTGATCAAC
ATGAGCCTGGGTGGCCCGAGCGGCAGCGCGGCGCTGAAGGCGGCGGTGGATAAAGCGGTTGCGAGCGGTGTGGTT
GTTGTGGCGGCGGCGGGTAACGAAGGCACCAGCGGTGGCAGCAGCACCGTGGGTTATCCGGGTAAATATCCGAGC
GTTATTGCGGTGGGTGCGGTTAACAGCAGCAACCAGCGTGCGAGCTTTAGCAGCGTTGGCAGCGAACTGGATGTG
ATGGCGCCGGGTGTTAGCATCCAAAGCACCCTGCCGGGTAACAAGTACGGCGCGTATAACGGCACCAGCATGGCG
AGCCCGCACGTGGCGGGTGCTGCGGCGCTGATTCTGAGCAAACACCCGAACTGGACCAACACCCAGGTTCGTAGC
AGCCTGGAAAACACCACCACCCGTCTGGGCGATGCGTTTTACTATGGCAAAGGTCTGATCAATGTTCAAGCGGCG
GCGCAG

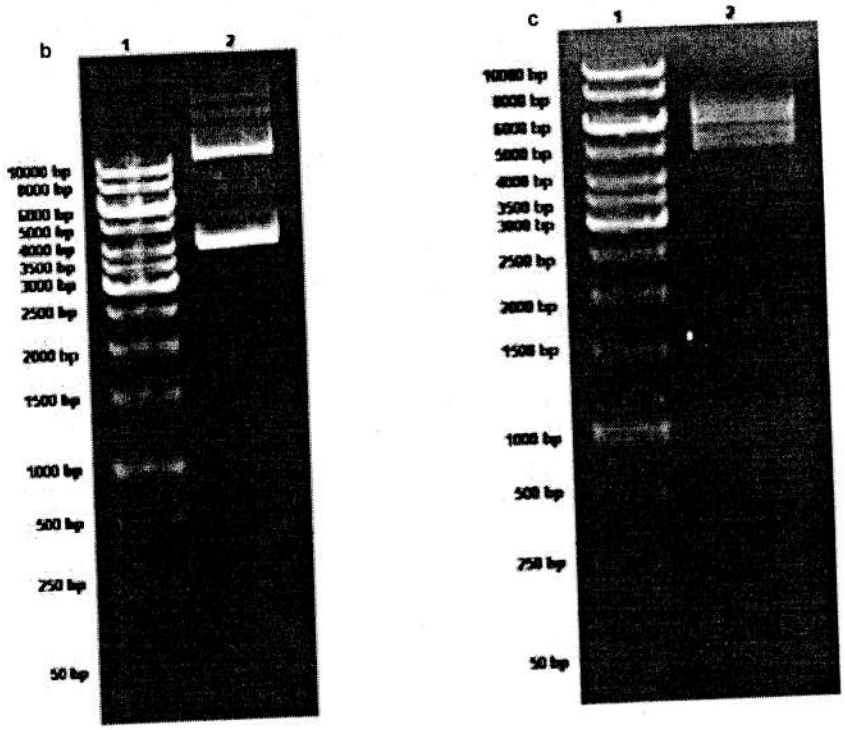


Fig.1B. a. Optimized sequence. b. Plasmid isolation of recombinat clone; Lane1, DNA marker; Lane2, isolated recombinant plasmid. c. Restriction digestion of isolated recombinat plasmid with NcoI and XhoI enzymes; Lane1, DNA marker; Lane2, RE digested product.

2. Biochemical and pharmacological characterization of purified recombinant protease, and comparison with commercial anticoagulant/fibrinogenolytic drugs:

2A. Induction, isolation and purification of recombinant protein (protease enzyme)

The transformed recombinant cell colonies were allowed to grow in 5 ml LB broth supplemented with Kanamycin for 12 h. After that the cell culture was inoculated in 100 ml LB broth with Kanamycin and incubated at 37°C. When the OD_{600} of the cell culture reached 0.6, protein expression was induced by 1 mM of IPTG followed by incubation for another 24 h. The induced cells were harvested by centrifugation, the pellet was resuspended in bacterial cell lysis buffer, and lysed by sonication. After centrifugation, the supernatant (soluble fraction) did not show any fibrinogenolytic activity which was analyzed by SDS-PAGE. The cell pellet (insoluble fraction containing inclusion bodies) was further processed by re-suspending it in 20 mM Tris-HCl buffer (pH 8.0) containing 8 M urea and dialysed against 20 mM Tris-HCl buffer (pH 8.0) containing 6 M urea using dialysis sacks having flat width 30 mm, 12000 Da at 4°C for 18 h. The concentration of urea was being reduced from 6 M-4 M-2 M-1 M-0 M by changing the buffer at an

interval of 2 h and final dialysis with 0 M concentration of urea was continued for 10 h. After the completion of dialysis the cells were harvested by centrifugation and the supernatant was used for further analysis.

The expression of desired protein was confirmed by mixing ~50 µg of supernatant with reducing dye and separated by 12.5% SDS-PAGE. The protease bands were detected by phast stain. A sharp band was visualized in the gel and after calculating the R_f value, it was estimated to be ~42 kDa (Fig.2A-a). The expressed protein was further analysed by western blot using His-tag antibody that confirmed the expression of recombinant proteins (Fig.2A-b).

The supernatant containing the expressed protein was passed through Ni-NTA affinity column previously equilibrated with lysis buffer. The flow through was discarded and the column was washed with 10 ml lysis buffer. The protein of interest bound in the column was eluted by stepwise increase in imidazole concentration of the elution buffer starting from 100, 200, 250, 300, 500 and 750 mM. A 12.5% SDS-PAGE was run to ensure the elution of protein of interest from each concentration of imidazole fraction. A band was detected from the 250 mM imidazole fraction at ~42 kDa. Fibrinogenolytic assay was initiated by incubating ~20 µg of 250 mM imidazole fraction with fibrinogen for 90 min at 37°C. The treated sample after incubation were analysed by 12.5% SDS-PAGE where it showed degradation of Aα-chain of fibrinogen but the Bβ and γ-chains were not degraded, indicating that the expressed protein have a moderate Aα-fibrinogenase activity (Fig.2A-c).

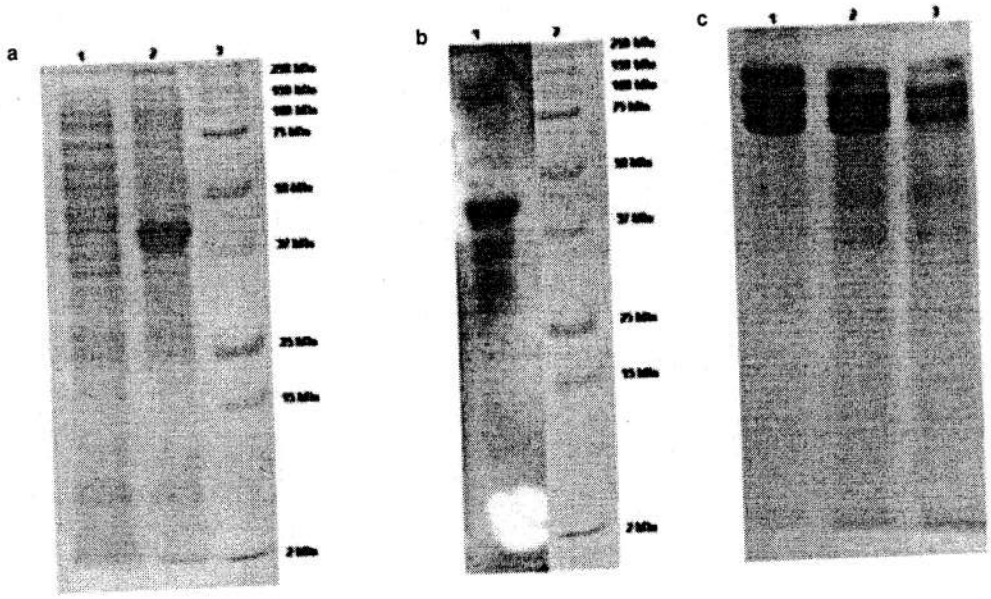


Fig.2A. a. 12.5% SDS-PAGE of expressed protein; Lane1, Insoluble fraction; Lane2, Soluble fraction after dialysis;Lane3 Protein marker. B. Western blot by His-tag antibody; Lane1, Soluble fraction after dialysis; Lane2, Protein marker. C. 12.5% SDS-PAGE for fibrinogenolytic assay;Lane1, Negative control (Fibrinogen); Lane2, Positive control (Nattokinase); Lane3, Ni-NTA purified fraction

II. National institute of Pharmaceutical Education and research (NIPER)

3. Improvement of fibrinogen binding potency of recombinant fibrin(ogen)olytic serine protease for enhancing its catalytic activity:

Process optimization of the fermentation conditions for the growth and production of fibrinolytic enzymes by recombinant *E. coli* was carried out in shake flask. Glucose and malt extract have been found to be optimum carbon and nitrogen sources, respectively. Further optimization of parameters affecting the fermentation conditions is being carried out by one factor at a time as well as by statistical approach. Fibrinolytic enzyme was produced by culturing *E. coli* in conical flask in shaking condition. Experiments were carried out in 50 mL medium in 250 mL Erlenmeyer flasks. The medium was inoculated with 2% inoculum and the flasks were incubated at 37°C and 200 rpm for 15 h with monitoring of the cell growth at specific time interval. The growth of microorganisms in the culture broth is measured by taking optical density and plotting the growth curve as well as the standard curve of wet cell mass vs. absorbance. The reducing sugar content in the fermentation broth was measured by the DNS method. Experiments were carried out in Minimal and in Luria Bertani broth with various carbon sources such as maltose, lactose, sucrose, starch, cellulose, glycerol etc. Several nitrogen sources both organic as well as inorganic were

also tried. LB medium was found to be better in terms of cell growth yield than Minimal media. Among the various carbon sources used, glucose as a carbon source was found to have a maximum cell mass yield (39 mg/mL) followed by sucrose (26.27 mg/mL) and lactose (24.15 mg/mL) (Fig. 3a).

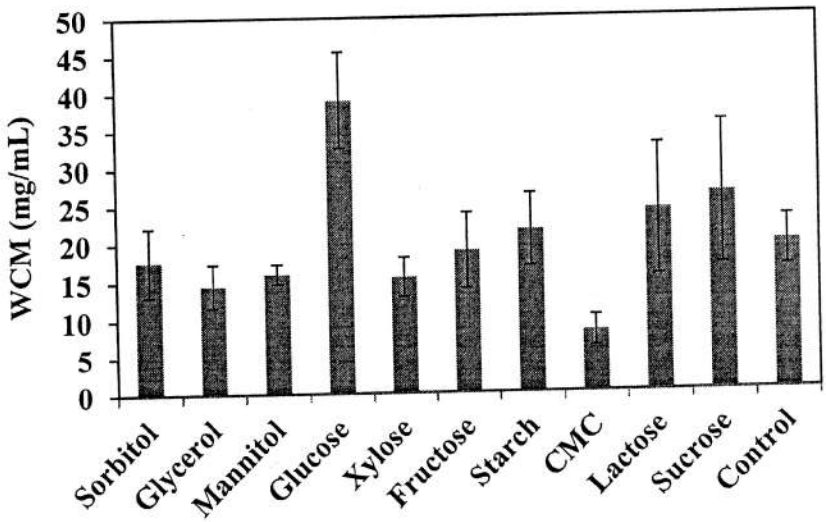


Fig 3a. Effect of different carbon sources on cell mass yield

Different nitrogen sources were screened and malt extract as a nitrogen source was found have a maximum cell mass yield (45.09 mg/mL) followed by yeast extract (30.9 mg/mL) and soya peptone (27.68 mg/mL) (Fig. 3b).

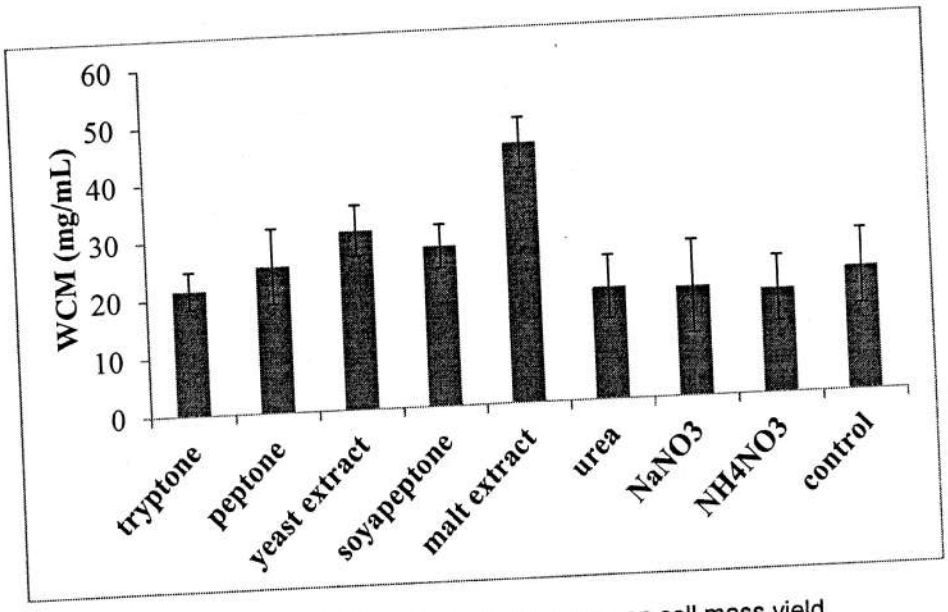


Fig 3b. Effect of different nitrogen sources on cell mass yield

(Ashis K. Mukherjee)

Date: 09/11/2020

(Uttam Chandra Banerjee)

Date:

Progress Report for R&D Projects [Year 2018-2019]

Section-A: Project Details

A1. **Project Title:**Pharmacological studies of recombinant and mutant fibrin(ogen)olytic protease(s) for the prevention and/or treatment of hyperfibrinogenemia associated cardiovascular disorders.

A2. **DBT Sanction Order No. & Date:** 102/IFD/SAN/1809/2018-2019, Dated- 27.08.2018

A3. **Name of Principal Investigator:** Prof. A. K. Mukherjee, Tezpur University
Name of Co-PI/Co-Investigator: Dr. Suman Das Gupta, T.U.

A4. **Institute:** Tezpur University,Tezpur(Assam)

A5. **Address with Contact Nos. (Landline & Mobile) & Email:** Department of Molecular Biology & Biotechnology, Tezpur University, School of Science and Technology, Tezpur-784028, Contact No- 03712-267006, 7896003886 (M), Email- akm@tezu.ernet.in

A6. **Total Cost:** Rs. 19,19,600.00 (Rupees Nineteen lakh nineteen thousand six hundred only) (For Tezpur University only)

A7. **Duration:** 1.5 Years (2018-2019)

Section-B: Scientific and Technical Progress: Shown as Annex-I

B1. **Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period** (1000-1500 words for interim reports; 2500-3500 words for final report; data must be included in the form of up to 3 figures and/or tables for interim reports; up to 7 figures and/or tables for final reports):

B2. **Summary and Conclusions of the Progress made so far** (minimum 100 words, maximum 200 words): Fibrinogenolytic enzyme producing Gram positive *Bacillus sp.* was isolated from fermented food sample of NE India and its genomic DNA was isolated by GeneJET genomic DNA purification kit. The appropriate primer set to amplify the fibrinogenolytic gene from this bacterium were designed and the desired fibrinogenolytic encoding gene sequence was PCR amplified. The gene sequence was confirmed by DNA sequencing. This was followed by synthesis of protease gene by codon optimization.

B3.**Connectivity of the partnering institutes (Institute wise achievements to be given separately for each objective).**

B4. **Details of New Leads Obtained, if any:** No.

B5. **Details of Publications & Patents, if any:** No.

B6. **The training undertaken by the NER PI and the recruited manpower at the Collaborating Institution/s** (Details of personnel trained, duration of training and training undertaken): No

B7. **The details of visits of the Collaborating institutes PI and personnel's**
(Purpose and duration of visits):

- Visit of PI to NIPER, Chandigarh from 07.01.2019 to 09.01.2019

Section-C: Details of Grant Utilization#

C1. Equipment Acquired or Placed Order with Actual Cost: Thermalcycler with accessories with a cost of Rs. 5,98,500.00 only.

C2. Manpower Staffing and Expenditure Details: One JRF joined on 21st December'2018 at a monthly salary of Rs.12000(+HRA). Total expenditure during the first year is Rs. 41,884.00 only.

C3. Details of Recurring Expenditure: Total recurring expenditureisRs.3,62,433.00only.

C4. Financial Requirements for the Next Year with Justifications: Sanction amount of Rs.6,84,800.00 for the next financial year(2019-2020).

[Signature(s) of the Investigator(s)]

Instructions:

(i) *All the information needs to be provided; otherwise the Progress Report will be treated as incomplete. In case of 'Nil' / 'Not Applicable' information, the same may be indicated.*

(ii) *In case of multicentre project, a combined Progress Report should be submitted incorporating the progress of all components. The Project Co-coordinator/ PI will be responsible for this.*

(iii) **Please indicate the reporting period [i.e. Year 1/2/3/4/5].*

(iv) *Submission of Progress Report by the end of the 11th month of grant sanction is linked with further continuation of the project and timely release of funds for the next year.*

Appendix-B

Utilization Certificate

(For the financial year ending 31st March, 2019)

1	Title of the Project/Scheme:	Pharmacological studies of recombinant and mutant fibrin(ogen)olytic protease(s) for the prevention and/or treatment of hyperfibrinogenemia associated cardiovascular disorders.
2	Name of the Organization:	Department of Molecular Biology & Biotechnology, Tezpur University.
3	Principal Investigator:	Prof. Ashis K. Mukherjee
4	Dept. of Biotechnology sanction order No. & date of sanctioning the project:	Sanction order no.- 102/IFD/SAN/1809/2018-2019 Dated- 27.08.2018
5	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	Not applicable
6	Amount received from DBT during the financial year (2018-2019) :	₹ 19,19,600.00
7	Other receipts/interest earned, if any, on the DBT grants:	₹ 0.00
8	Total amount that was available for expenditure during the financial year:	₹19,19,600.00
9	Actual expenditure (excluding commitments) incurred during the financial year 2018-2019 (statement of expenditure is enclosed)	₹3,62,433.00
10	Unspent balance refunded, if any *(Please give details of cheque No. etc.)*:	NA
11	Balance amount available at the end of the financial year 2018-2019 (excluding commitments expenditure):	₹ 15,57,167.00
12	Amount allowed to be carried forward to the next financial year vide letter No. & date:	₹15,57,167.00

1. Certified that the amount of **₹3,62,433.00**(Rupees three lakh sixty two thousand four hundred thirty three only) mentioned against col. 9 has been utilized on the project/scheme for the purpose for which it was sanctioned and that the balance of **₹15,57,167.00** (Rupees fifteen lakh fifty seven thousand one hundred sixty seven only) remaining unutilized at the end of the year ~~has been surrendered to Govt. (vide DD no. _____ dated _____) will be adjusted towards the grants-in-aid payable during the next year~~. **may kindly be allowed to utilized in the financial year 2019-2020.**

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Equipments were procured following Central govt. purchase rules
2. Chemicals were purchased from T&PC approved vendors and approval rules
3. Travel grant was utilized for the purpose for which it was granted
4. Contingency grant was also utilized following the rule
5. Items purchased from equipment, chemical/consumable, contingency and travel head were entered in stock register

(PROJECT INVESTIGATOR)

(FINANCE OFFICER)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)

(To be countersigned by the DBT Officer-in-charge)

Registrar
Tezpur University

Statement of Expenditure referred to in para 9 of the **Utilization Certificate**

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from **7th September, 2018 to 31th March, 2019.**

Items	Heads	Unspend balance carried forward from previous year (₹)	Grant received from DBT during the year (Sep'2018-March'2019) (₹)	Other receipts / Interest earned if any, on the DBT grant (₹)	Total (2+3+4) (₹)	Expenditure (excluding commitments) incurred during the year (2018-2019) (₹)	Balance (5-6) (₹)	Remar
	1	2	3	4	5	6	7	8
1. Non - recurring								
(i)	Equipments	0.00	6,00,000.00		6,00,000.00	0.00	6,00,000.00	
2. Recurring								
(i)	Human resource	0.00	3,69,600.00	0.00	3,69,600.00	41,884.00	3,27,716.00	
(ii)	Chemical and Consumable	0.00	8,00,000.00		8,00,000.00	2,14,415.00	5,85,585.00	
(iii)	Travel	0.00	50,000.00		50,000.00	40,377.00	9,623.00	
(iv)	Contingency	0.00	50,000.00		50,000.00	34,507.00	15,493.00	
(v)	Overhead	0.00	50,000.00		50,000.00	31,250.00	18,750.00	
Totals		**0.00**	**19,19,600.00**	**0.00**	**19,19,600.00**	**3,62,433.00**	**15,57,167.00**	

(PROJECT INVESTIGATOR)

(FINANCE OFFICER)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

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Progress Report for R&D Projects [Year 2019-2020]

Section-A: Project Details

A1. **Project Title:** Pharmacological studies of recombinant and mutant fibrin(ogen)olytic protease(s) for the prevention and/or treatment of hyperfibrinogenemia associated cardiovascular disorders.

A2. **DBT Sanction Order No. & Date:** 102/IFD/SAN/1809/2018-2019, Dated- 27.08.2018

A3. **Name of Principal Investigator:** Prof. A. K. Mukherjee, Tezpur University
Name of Co-PI/Co-Investigator: Dr. Suman Das Gupta, T.U.

A4. **Institute:** Tezpur University,Tezpur(Assam)

A5. **Address with Contact Nos. (Landline & Mobile) & Email:** Department of Molecular Biology & Biotechnology, Tezpur University, School of Science and Technology, Tezpur-784028, Contact No- 03712-267006, 7896003886 (M), Email- akm@tezu.ernet.in

A6. **Total Cost:** Rs. 19,19,600.00 (Rupees Nineteen lakh nineteen thousand six hundred only) (For Tezpur University only)

A7. **Duration:** 1.5 Years (2018-2019)

Section-B: Scientific and Technical Progress: Shown as Annex-I

B1. **Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period** (1000-1500 words for interim reports; 2500-3500 words for final report; data must be included in the form of up to 3 figures and/or tables for interim reports; up to 7 figures and/or tables for final reports):

B2. **Summary and Conclusions of the Progress made so far** (minimum 100 words, maximum 200 words): The synthetic gene containing pro-peptide and mature enzyme sequence was cloned in between the NcoI and XhoI site of pET26b(+) expression vector. The recombinant pET26b(+) vector was transformed into BL21a competent cells and spreaded on LB agar plates containing kanamycin. For the proper confirmation of transformation, plasmid isolation was done from all colonies of each plate followed by double digestion. The confirmed transformed cells were grown in LB broth media containing kanamycinat 37°C until the cell density reached 0.4-0.5 at 620 nm. Protein expression was induced with 1 mM IPTG followed by growing the cells for another 24 hr at 37°C. The induced protein expression was confirmed by SDS-PAGE gel and purified with Ni-NTA column. The isolated recombinant protein showed fibrinogenolytic activity by SDS-PAGE analysis; however, the activity was found to be transient and warrants to find out the mechanism to stabilize the enzyme.

B3. **Connectivity of the partnering institutes (Institute wise achievements to be given separately for each objective).**

B4. **Details of New Leads Obtained, if any:** No.

B5. **Details of Publications & Patents, if any:** No.

B6. **The training undertaken by the NER PI and the recruited manpower at the Collaborating Institution/s** (Details of personnel trained, duration of training and training undertaken): No

B7. **The details of visits of the Collaborating institutes PI and personnel's** (Purpose and duration of visits): No

Section-C: Details of Grant Utilization#

C1. Equipment Acquired or Placed Order with Actual Cost: Thermalcycler with accessories with a cost of Rs. 5,98,500.00 only.

C2. Manpower Staffing and Expenditure Details: One JRF joined on 21st December'2018 at a monthly salary of Rs.12000(+HRA). Total expenditure during the second year is Rs. 1,10,516.00only.

C3. Details of Recurring Expenditure: Total recurring expenditureisRs. 6,84,323.00 only.

C4. Financial Requirements for the Next Year with Justifications: NA

[Signature(s) of the Investigator(s)]

Instructions:

(i) *All the information needs to be provided; otherwise the Progress Report will be treated as incomplete. In case of 'Nil' / 'Not Applicable' information, the same may be indicated.*

(ii) *In case of multicentre project, a combined Progress Report should be submitted incorporating the progress of all components. The Project Co-coordinator/ PI will be responsible for this.*

(iii) **Please indicate the reporting period [i.e. Year 1/2/3/4/5].*

(iv) *Submission of Progress Report by the end of the 11th month of grant sanction is linked with further continuation of the project and timely release of funds for the next year.*

Appendix-B

Utilization Certificate

(For the financial year ending 31st March, 2020)

1	Title of the Project/Scheme:	Pharmacological studies of recombinant and mutant fibrin(ogen)olytic protease(s) for the prevention and/or treatment of hyperfibrinogenemia associated cardiovascular disorders.
2	Name of the Organization:	Department of Molecular Biology & Biotechnology, Tezpur University.
3	Principal Investigator:	Prof. Ashis K. Mukherjee
4	Dept. of Biotechnology sanction order No. & date of sanctioning the project:	Sanction order no.- 102/IFD/SAN/1809/2018-2019 Dated- 27.08.2018
5	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	₹ 15,57,167.00
6	Amount received from DBT during the financial year (2019-2020) :	₹ 0.00
7	Other receipts/interest earned, if any, on the DBT grants:	₹ 19,198.00
8	Total amount that was available for expenditure during the financial year:	₹15,76,365.00
9	Actual expenditure (excluding commitments) incurred during the financial year 2019-2020 (statement of expenditure is enclosed)	₹12,82,823.00
10	Unspent balance refunded, if any *(Please give details of cheque No. etc.)*:	NA
11	Balance amount available at the end of the financial year 2019-2020 (excluding commitments expenditure):	₹ 2,93,542.00
12	Amount allowed to be carried forward to the next financial year vide letter No. & date:	NA

1. Certified that the amount of **₹12,82,823.00**(Rupees twelve lakh eighty two thousand eight hundred twenty three only) mentioned against col. 9 has been utilized on the project/scheme for the purpose for which it was sanctioned and that the balance of **₹2,93,542.00**(Rupees two lakh ninety three thousand five hundred fourty two only) remaining unutilized at the end of the year has been surrendered to Govt. (vide DD no. _____ dated _____) will be adjusted towards the grants-in-aid payable during the next year.

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Equipments were procured following Central govt. purchase rules
2. Chemicals were purchased from T&PC approved vendors and approval rules
3. Travel grant was utilized for the purpose for which it was granted
4. Contingency grant was also utilized following the rule
5. Items purchased from equipment, chemical/consumable, contingency and travel head were entered in stock register

(PROJECT INVESTIGATOR)

(FINANCE OFFICER)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)

(To be countersigned by the DBT Officer-in-charge)

Registrar
Tezpur University

Statement of Expenditure referred to in para 9 of the **Utilization Certificate**

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from **1st April, 2019 to 31th March, 2020.**

Items	Heads	Unspend balance carried forward from previous year (₹)	Grant received from DBT during the year (April'2019-March'2020) (₹)	Other receipts / Interest earned if any, on the DBT grant (₹)	Total (2+3+4) (₹)	Expenditure (excluding commitments) incurred during the year (April'2019-March'2020) (₹)	Balance (5-6) (₹)	Remar
	1	2	3	4	5	6	7	8
1. Non - recurring								
(i)	Equipments	6,00,000.00	0.00		6,00,000.00	5,98,500.00	1,500.00	
2. Recurring								
(i)	Human resource	3,27,716.00	0.00		3,27,716.00	1,10,516.00	2,17,200.00	
(ii)	Chemical and Consumable	5,85,585.00	0.00	**19,198.00**	5,85,585.00	5,55,188.00	30,397.00	
(iii)	Travel	9,623.00	0.00		9,623.00	6,642.00	2,981.00	
(iv)	Contingency	15,493.00	0.00		15,493.00	11,977.00	3,516.00	
(v)	Overhead	18,750.00	0.00		18,750.00	0.00	18,750.00	
Totals		**15,57,167.00**		**19,198.00**	**15,76,365.00**	**12,82,823.00**	**2,93,542.00**	

(PROJECT INVESTIGATOR)

(FINANCE OFFICER)
Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)
Registrar
Tezpur University