APPENDIX I

(A) Screening media for fibrin(ogen)olytic enzyme(s) producing bacteria

Composition	Concentration (gm/l)
Fibrin	4.0
Yeast extract	2.5
Glucose	1.0
Agar	20.0
pH	8.0/10.0
Distilled water	1000 ml

Note: Adjust the media pH separately followed by addition of skim milk, agar and yeast extract.

(B) Composition of M9 Media for protease production

(I) M9 Medium composition	Concentration (gm/l)
Na_2HPO_4	6.0
KH_2PO_4	3.0
NH_4Cl	1.0
NaCl	0.5
$MgSO_4.H_2O$	0.246
$CaCl_2.7H_2O$	0.014
Carbon source	1.0
(II) Macro-nutrient composition	
$FeSO_4.7H_2O$	1.0 mg l^{-1}
$CuSO_4.5H_2O$	50.0 µg l^{-1}
H_3BO_3	10.0 µg l^{-1}
$MgSO_4.5H_2O$	10.0 µg l^{-1}
$ZnSO_4.7H_2O$	70.0 µg l^{-1}
MoO_3	10.0 µg l^{-1}

Add 1ml of macro –nutrient in 1000 ml of M9 production medium

(C) Carbohydrate fermentation medium compositions

(i) Phenol red broth	Concentration (gm/l)
Protease peptone	10.0
Beef extract	1.0
Sodium chloride	5.0
Sucrose/Lactose/fructose/D-glucose/ Mannitol	5.0
Phenol red	0.018

(D) Nutrient Agar composition	Concentration (gm/l)
Peptic digest of animal tissue	10.0
Meat extract	10.0
NaCl	5.0
Agar	15.0

(E) Litmus milk composition	Concentration (gm/l)
Skim milk powder	100.0
Litmus	0.50
Sodium sulphite	0.50

(F) Urea broth	Concentration (gm/l)
Yeast extract	0.1
Monopotassium phosphate	9.1
Dipotassium phosphate	9.5
Urea	20.0
Phenol red	0.01

(G) Tryptone broth	Concentration (gm/l)
Casein enzymatic hydrolysate	10.0

Sodium chloride	5.0

(H) Simmon citrate agar (SIM agar)	Concentration (gm/l)
Magnesium sulphate	0.20
Ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
NaCl	5.0
Bromothymol blue	0.08
Agar	15.0

(I) Nitrate broth	Concentration (gm/l)
Peptic digest of animal tissue	5.0
Meat extract	3.0
Potassium nitrate	1.0
NaCl	30.0

(J) Indole nitrate broth	Concentration (gm/l)
Casein enzymic hydrolysate	20.0
Disodium phosphate	2.0
Dextrose	1.0
Potassium nitrate	1.0
Agar	1.0

(K) Methyl red –voges proskauer broth	Concentration (gm/l)
Buffered peptone	7.0
Dextrose	5.0
Dipotassium phosphate	5.0

(L) Triple sugar iron agar	Concentration (gm/l)
Peptic digest of animal tissue	10.0
Casein enzymic hydrolysate	10.0
Yeast extract	3.0
Beef extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferric ammonium citrate	0.30
NaCl	5.0
Sodium thiosulphate	0.30
Phenol red	0.024
Agar	12.0

(M) Nutrient Broth	Concentration (gm/l)
Peptic digest of animal tissue	10.0
Meat extract	10.0
NaCl	5.0

(N)SDS-PAGE gel electrophoresis composition

(i) Resolving buffer (8X)	Concentration (gm/l)
1.5 M Tris-Cl (pH 8.8)	18.17

(ii) Stacking buffer (4X)	Concentration (gm/l)
0.5M Tris-Cl (pH 6.8)	6.06

Note : Adjust the pH 8.8 with 6N HCl. Make up the final volume to 100 ml with distilled water

(iii) Acrylamide mixture	Concentration (gm/l)
30 % acrylamide	30.0

0.8% bis-acrylamide	0.8

Note: Dissolve in 100ml of warm deionized water to facilitate dissolution of bis-acrylamide. Store in amber colour bottle at 4ºC

(iv) Reservoir buffer (pH 8.3, 1X)	Concentration (gm/l)
Tris-Cl	3.0
Glycine	14.4
10% SDS	10.0

Note : Dissolve in 1000ml of distilled water adjusted to pH to 8.3

(v) Sodium dodecyl sulfate (SDS)	Concentration (gm/l)
10 % SDS	10.0

(vi) Ammonium per sulphate (APS)	Concentration (gm/l)
10% APS	0.2

(vii) Staining solution	Volume (ml / concentration)
Methanol	40.0
Glacial acetic acid	10.0
Distilled water	50.0
Commassie brilliant blue	0.4 (gm/ ml)

(viii) Destaining solution

Composition	Volume (ml)
Methanol	40
Glacial acetic acid	10
Distilled water	50

(ix) Loading buffer (50 ml , 3X)

Composition	Amount / volume

Tris-Cl (pH 6.8)	1.296
SDS	3.0
Glycerol	1.5 ml
Bromophenol blue	3.0

Note : 1ml of loading buffer mixed with 30µl of 2-mercaptoethanol (3%)

(x) Resolving gel composition (12.5%)

Components	Volume (ml)
Distilled water	2.3
1.5 M Tris-Cl (pH 8.8)	5.0
Acrylamide mixture	8.3
10% SDS	0.2
1 % APS	1.5
Glycerol	0.8
TEMED	0.025

(xi) Stacking gel (4X)

Components	Volume (ml)
Distilled water	5.4
0.5M Tris-Cl (pH 6.8)	2.5
10% SDS	0.1
Acrylamide mixture	1.3
1 % APS	0.7
Glycerol	0.4
TEMED	0.015

(O) Chemicals composition for chromosomal DNA isolation

(i) Solution I	Concentration (gm %)
50mM glucose	0.9

25mM Tris-Cl	0.30
10mM EDTA	0.37

(ii) Solution II	Concentration (gm %)
0.2N NaOH	0.0079
SDS	1.0

(iii) Solution III	Volume (ml)
5M Potassium acetate	60.0
Glacial acetic acid	11.5
Distilled water	28.5

(iv) Loading dye	Concentration (gm %)
Bromophenol blue	0.25
Xylene cyanol	0.25
Sucrose	40.0

APPENDIX II

LIST OF PUBLICATIONS FROM Ph.D THESIS

1. **Sourav Majumdar**, Biplob Sarmah, Debanand Gogai, Subhamoy Banerjee, Siddhartha S.Ghosh, Pronobesh Chattopadhyay and Ashis K. Mukherjee, Characterization, mechanism of anticoagulant action, and assessment of therapeutic potential of a fibrinolytic serine protease (Brevithrombolase) purified from *Brevibacillus brevis* strain FF02B. *Biochimie* 2014, 103, 50-60.
2. **Sourav Majumdar** and Ashis K. Mukherjee, Biomedical application of Bacterial fibrinolytic protease as a thrombolytic agent for the coming era. *TSI Newsletter* 2012, 2, 39.
3. **Sourav Majumdar**, Samanwita Goswami, Chenole Keppen, Sudhir K. Rai and Ashis K. Mukherjee, Statistical optimization for improved production of fibrin(ogen)olytic enzyme by *Bacillus cereus* strain FF01 and assessment of *in vitro* thrombolytic potential of protease enzyme. *Biocatalysis and Agricultural Biotechnology* 2014, http://dx.doi.org/10.1016/ j.bcab.2014.11.004.
4. **Sourav Majumdar**, Pronobesh Chattopadhyay and Ashis K. Mukherjee, *In vivo* anticoagulant and thrombolytic activities of a fibrinolytic serine protease (Brevithrombolase) with the k-carrageenan-induced rat tail thrombosis model. *Clinical and applied Thrombosis/Hemostasis* 2015, doi: 10:1177/1076029615569567.

MANUSCRIPTS COMMUNICATED

1. **Sourav Majumdar,** Sumita Dutta, Tanushree Das, Ashis K. Mukherjee , Assessment of *in vivo* thrombolytic potency, anticoagulant efficacy, and antiplatelet effect of a non-toxic, non-hemorrhagic new fibrin(ogen)olytic serine protease (Bacethrombase) purified from *Bacillus cereus* strain FF01 (under review).

PAPER PRESENTED IN SEMINAR / CONFERENCES FROM Ph.D. THESIS

1. **Sourav Majumdar** and Ashis K. Mukherjee, Characterization and pharmaceutical application of a potent fibrin(geno)lytic enzyme purified from Bacillus sp. FF02B strain isolated from fermented food of NE India, proceeding in Recent Advances in Microbial Biotechnology and Molecular Evolution at Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur on 1st to 4th March, 2013.
2. Biplob Sarmah, **Sourav Majumdar** and Ashis K. Mukherjee, Bacterial fibrinolytic protease can be a promising source of potent thrombolytic agent in near future, proceeding in Recent Advances in Microbial Biotechnology and Molecular Evolution at Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur on 1st to 4th March, 2013.
3. **Sourav Majumdar,** Samanwita Goswami, Chenole Keppen and Ashis K. Mukherjee, Statistical optimization and purification of a fibrin(ogen)olytic enzyme produced by *Bacillus cereus* strain FF01: assessment of its pharmacological potential, proceeding in 25th Annual conference, North east regional chapter of Indian association of pathologist and microbiologist at Department of Pathology, Naga hospital authority Kohima, Nagaland on 20th -21st September 2014.

4. **Sourav Majumdar** and Ashis K. Mukherjee , Screening a fibrinolytic enzyme producing *Brevibacillus brevis* strain FF02Bfrom traditional fermented foods of North-East India and its pharmacological applications, proceeding in 55th Annual Conference, National Conference on Empowering Mankind with Microbial Technologies organized by Association of Microbiologists of India, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore - 641 003, November 12th – 14th, 2014.
5. **Sourav Majumdar** and Ashis K. Mukherjee, Pharmacological characterization and mechanism of anticoagulant action of fibrinolytic serine protease purified from *Brevibacillus brevis*, proceeding in Recent Advances in Microbial Biotechnology and Molecular Evolution at Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur on 27th-29th November, 2014.
6. **Sourav Majumdar** and Ashis K. Mukherjee, A bacterial fibrinolytic enzyme (Brevithrombolase) with unique anticoagulant mechanism from *Brevibacillus brevis* strain FF02B, 102nd Indian Science Congress, 3rd - 7th January 2015 at University of Mumbai, Mumbai.