Data on isolation and purification of fibrinolytic enzyme from Pseudomonas baetica SUHU25

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ABSTRACT

The present dataset provides methodology to isolate and purify fibrinolytic enzyme from microbe isolated from the natural source. The information provided in this data article includes (1) isolation and identification of Pseudomonas baetica SUHU25, (2) optimization of cultural conditions, (3) extraction and purification of fibrinolytic enzyme, (4) protein estimation, (5) assay of fibrinolytic activity, (6) SDS PAGE for purified enzyme protein, (7) effect of pH, temperature and metal ions on fibrinolytic activity of enzyme protein, and (8) In-vitro blood clot dissolution assay.

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1. Data

Pseudomonas baetica SUHU25 isolated from a local fish market showed proteolytic activity on skim milk agar as presented in Fig. 1 and fibrinolytic activity on fibrin agar in Fig. 2. Fig. 3 shows the Phylogenetic tree of Pseudomonas baetica SUHU25 obtained after 16s rRNA sequencing. Figs. 4–8
shows relative activity (%) of fibrinolytic enzyme of *Pseudomonas baetica* SUHU25 with change in carbon source, nitrogen source, pH, temperature and incubation period respectively. Fig. 9 shows fibrinolytic enzyme activity of cell free media supernatant of *Pseudomonas baetica* SUHU25. Fig. 10 represents the SDS PAGE of purified fibrinolytic enzyme from *Pseudomonas baetica* SUHU25. Fig. 11 shows In-vitro blood clot dissolution by the purified enzyme preparation. Figs. 12–14 shows relative activity (%) of fibrinolytic enzyme with change in pH, temperature and presence of various metals respectively. Purification scheme of fibrinolytic enzyme form *Pseudomonas baetica* SUHU25 is detailed in Table 1.

2. Experimental design, materials, and methods

2.1. Screening for fibrinolytic microorganisms

Sample was collected in sterile container from a local fish market at wash and waste disposal site in Pune, India for the isolation of potential fibrinolytic microbes. Primary Screening was done by serially diluting sample with physiological saline up to $10^{-3}$. 0.1 ml of dilution was plated on nutrient agar plates and incubated at 37 °C for 24 hours for microbial growth. Each well isolated bacterial colony after 24 hours of growth on nutrient agar was spot inoculated on skim milk agar for analyzing proteolytic activity. Cultures showing zone of clearance on skim milk agar were further spot inoculated on fibrin agar for 24 hours at 37 °C for secondary screening. Zone of clearance was noted after incubation for each culture [1].
2.2. Identification of fibrinolytic isolate

Isolated culture showing larger zone of clearance on fibrin agar plate was identified by 16s rRNA Sequencing. Genomic DNA was extracted from the isolates using BioEra’s Genomic DNA extraction kit. 16s rDNA gene was amplified by forward primer 5’- AGAGTRTGATCMTYGCTWAC-3’ and reverse primer 5’CGYTAMCTTWTTACGRCT-3’ with the programme consisting of denaturation at 94 °C for 5 minutes and subsequent 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 2 minutes followed by final extension at 72 °C for 5 minutes. The sequence analysis was performed using the ABI 3130 genetic analyzer and Big Dye Terminator version 3.1 cycle sequencing kit. The amplified product sequence comparison with database was performed using BLAST through the NCBI server [2].
2.3. Optimization of cultural conditions

The isolated culture was grown in the mineral salt medium (g/L: KH$_2$PO$_4$, 0.42; K$_2$HPO$_4$, 0.375; (NH$_4$)$_2$SO$_4$, 0.244; NaCl, 0.015; CaCl$_2$.2H$_2$O, 0.015; MgSO$_4$.7H$_2$O, 0.05; and FeCl$_3$.6H$_2$O, 0.054; pH 7 ± 0.1) supplemented with different carbon sources (such as 1% w/v glucose, fructose, maltose, sucrose, lactose and trehalose) and nitrogen sources (such as 1% w/v peptone, yeast extract, casein, beef extract, gelatin, ammonium chloride and ammonium sulphate). Effect of various physical parameters such as initial pH (4, 5, 6, 7, 8 and 9), temperature (20, 25, 30,
37, and 40 °C) and incubation period (24, 48, 72, and 96 hours) were checked. Optimization of variables was done by one-variable-at-a-time approach. All the experiments were conducted in triplicates.

2.4. Extraction and purification of fibrinolytic enzyme

The isolated culture was grown at 37 °C for 24 hours in the optimized nutrient medium (g/L: glucose, 10; casein, 10; KH2PO4, 0.42; K2HPO4, 0.375; NaCl, 0.015; CaCl2.2H2O, 0.015; MgSO4.7H2O, 0.05; and FeCl3.6H2O, 0.054; pH 6 ± 0.1). After incubation cells were separated from nutrient medium by centrifugation at 6000 rpm for 15 minutes at 4 °C. The fibrinolytic enzyme protein was

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**Fig. 4.** Effect of different carbon source on fibrinolytic activity of *Pseudomonas baetica* SUHU25.

**Fig. 5.** Effect of different nitrogen source on fibrinolytic activity of *Pseudomonas baetica* SUHU25.
extracted from the cell free supernatant by precipitation with five volumes of ice-cold acetone. Fibrinolytic enzyme from the acetone precipitated proteins was purified by anion exchange resin (DEAE Sephadex A50) and gel filtration chromatography on sephadex G100. All the steps were performed at 4 °C.

2.5. Protein estimation

Protein was estimated using dye-binding method described by Bradford [3].

2.6. Assay of fibrinolytic activity

Fibrinolytic activity was determined as reported by Tharwat et al. [4].
2.7. SDS PAGE for purified enzyme protein

SDS-PAGE was carried out to determine the purity and molecular weight of enzyme protein as described by Laemmli [5] using 10% polyacrylamide resolving gel. The gel was stained with Coomassie Brilliant Blue G 250 to visualize the protein bands.

2.8. Effect of pH and temperature on fibrinolytic activity of enzyme protein

The optimal pH for fibrinolytic activity of the enzyme was determined within pH range of 3–10 using acetate buffer (0.1 M, pH 3, 4 and 5), phosphate buffer (0.1 M, pH 6, 7 and 8), and Glycine buffer (0.1 M, pH 9 and 10). The effect of temperature was determined by measurement of residual activity of enzyme after incubation at different temperatures (4, 10, 20, 25, 30, 37, 40, 50 and 60 °C). 1 mg ml⁻¹ of

Fig. 8. Effect of variable incubation period on fibrinolytic activity of Pseudomonas baetica SUHU25.

Fig. 9. Fibrinolytic activity on fibrin agar plate by cell free media supernatant of Pseudomonas baetica SUHU25 grown in optimized cultural conditions.
Fig. 10. SDS PAGE of purified fibrinolytic enzyme from *Pseudomonas baetica* SUHU25. Lane 1 – Molecular weight marker; Lane 2 – Purified enzyme protein.

Fig. 11. Lysis of Blood clot in-vitro by purified fibrinolytic enzyme preparation of *Pseudomonas baetica* SUHU25.
purified enzyme preparation was used for all reactions. The activity of enzyme was assayed as reported by Tharwat et al. [4].

2.9. Effect of metal ions on fibrinolytic activity of enzyme protein

The effect of presence of metal ions was determined by using MgCl₂, ZnCl₂, FeCl₃, CuSO₄, CaCl₂, MnSO₄, and HgCl₂ at a final concentration of 10 mM. The purified preparation of enzyme at a
concentration of 1 mg ml\(^{-1}\) was pre-incubated in both the presence and absence of each cation for 1 hour at 37 °C. The activity of enzyme was assayed as reported by Tharwat et al. [4].

2.10. In-vitro blood clot dissolution assay

In vitro blood clot analysis was carried out according to Avhad et al. [6] with some modifications. 5 ml of purified enzyme preparation added to 0.5 g human blood clot in a test tube and kept for incubation at 37 °C till the complete blood clot lyses.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.104369.
References

[1] P.K. Aradhye, M.D. Chavan, Fibrinolytic effect of protease enzyme from Pseudomonas putida B-18, Int. J. Curr. Res. 9 (2) (2017) 46482–46487.

[2] S.F. Altschul, T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST, a new generation of protein database search programs, Nucleic Acids Res. 25 (1997) 3389–3402.

[3] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.

[4] N.A. Tharwat, Purification and biochemical characterization of fibrinolytic enzyme produced by thermophilic fungus Oidiodendron flavum, Biotechnology 5 (2) (2006) 160–165.

[5] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of Bacteriophage T4, Nature 227 (1970) 680–685.

[6] D.N. Avhad, S.S. Vanjari, V.K. Rathod, A novel fibrinolytic enzyme from Bacillus Sphaericus MTCC 3672: optimization and purification studies, Am. J. Curr Microbiol. 1 (1) (2013) 1–13.