## Application of Box-Behnken Design for the Optimization of Culture Conditions for Novel Fibrinolytic Enzyme Production by *Bacillus altitudinis* S-CSR 0020

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http://dx.doi.org/10.22207/JPAM.11.3.28

(Received: 20 June 2017; accepted: 25 August 2017)

A bacterium which produced novel extracellular fibrinolytic enzyme for digesting bovine blood clots was isolated from soil, and identified by 16s rRNA sequencing as *Bacillus altitudinis*, given strain name was S-CSR 0020 (accession number KT369312). Fibrin proved the best nitrogen source with an enzyme activity of 750 U/mL, followed by casein after incubation at 37 °C for 4 days. The cultural conditions were optimised using Response Surface Methodology (RSM) and Box-Behnken Design (BBD). Based on 3D surface plot and contour plots, the optimized temperature, pH and substrate concentrations were 47 °C, 10.5 and 4 g/L respectively, resulted in increase in enzyme activity of 306.88 U/mL and specific activity of 780 U/mg which was 2-fold; compared to initial level of 400 U/mg after 2 days of incubation. The crude enzyme has got potent activity and digested human blood clot completely within 1 hr.

> Keywords: *Bacillus altitudinis* S-CSR 0020, bovine blood clot, Box Behnken Design, fibrinolytic enzyme.

Fibrin plays a vital role in healing. Inappropriate clotting in blood vessel is a major factor for myocardial infarction and other cardiovascular diseases, resulted in 18 million of people dying every year as reported by World Health Organization. Fibrin, the major component involved in blood clot is formed from fibrinogen by proteolytic activity of thrombin. Meanwhile, plasmin hydrolyzed the clots to avoid thrombosis. But in an unbalanced situation, the fibrin clots are not hydrolyzed, and thrombosis occurs<sup>14</sup>. Twenty enzymes present in the body to assist in clotting of blood, while only the plasmin or any other plasmin like protease can break it down<sup>3</sup>. For the treatment of cardio-vascular diseases, various blood clotdissolving agents such as urokinase, streptokinase, and tissue plasminogen activator (t-PA) have been utilized<sup>24</sup>. Despite widespread use, these fibrinolytic agents suffer from various side effects including bleeding complications, short half-life, expensiveness, risk of anaphylactic reactions and large therapeutic doses<sup>4</sup>, so there is a necessary for the search of new fibrinolytic agents from various sources.

Various proteases from microorganisms have been purified and characterized which are having capability to interfere with the blood clotting mechanism. Microbial fibrinolytic enzymes are classified in to three types: Serine protease,

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