Arab J Sci Eng
DOI 10.1007/s13369-017-2475-5

RESEARCH ARTICLE - BIOLOGICAL SCIENCES

Application of Multifactorial Experimental Design for Optimization of Streptokinase Production Using *Streptococcus equisimilis* SK-6

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Received: 29 November 2016 / Accepted: 5 March 2017

Abstract The study involves statistical experimental designs for optimizing culture conditions to enhance streptokinase synthesis by *Streptococcus equisimilis* SK-6. One-factor-at-a-time method of optimization demonstrated variables (glucose, tryptone and period of incubation) influenced streptokinase production. Further, optimization under shake flask fermentation was performed using central composite design of response surface methodology. The value of R^2 (0.9403) indicated better association between the predicted and observed responses. Additionally, model F value of 17.50 and coefficient of variation (16.37) indicated the model to be significant ($p > 0.0001$), leading to a highly reliable experimental design. Compared to the unoptimized conditions, a 3.2-fold increase in streptokinase production (0.269 U/ml) by *S. equisimilis* SK-6 was observed within 48 h in a medium containing tryptone (18 g/l) and glucose (10 g/l). This study is possibly the first attempt to optimize streptokinase synthesis from a wild strain of *S. equisimilis* by use of multifactorial experimental design, and hence it can be regarded as a model approach for enhancing the produce of streptokinase by industrial fermentation process.

Keywords *Streptococcus equisimilis* SK-6 · Streptokinase · Optimization · CCD

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1 Introduction

Cardiovascular complications (ischemic stroke and acute myocardial infarction) are often associated with intravascular thrombus development. Other than surgical interventions, administration of thrombolytics namely streptokinase, urokinase and tissue plasminogen activator intravenously can be opted to clear intravascular fibrin clot [1,2]. Although all these proteins help in conversion of plasminogen to plasmin resulting in fibrinolysis and normal blood flow, urokinase and tissue plasminogen activator are known to have significantly reduced half-life besides being immunologically inert in nature. Therefore, streptokinase is the preferred biological agent for thrombolytic treatment [3].

Streptokinase is an extracellular protein produced by several naturally isolated forms of β-hemolytic streptococci inhabiting healthy humans and animals. The β-hemolytic group C streptococci are opted for streptokinase production since they are less fastidious in their growth requirements than the group A strains and do not secrete erythrogenic toxins [4]. Among group C streptococci, *Streptococcus equisimilis* has been widely used for streptokinase production since it yielded the most active streptokinase [5].

The exponential increase in the administration of streptokinase as therapeutic agent emphasizes on its qualitative improvement and quantitative enhancement [6]. Therefore, besides selecting a potent microbial culture, selection of medium components is equally vital for streptokinase production since microorganisms require optimum cultural conditions for growth and metabolite synthesis [7]. The traditional media optimization strategy involving one-factor-at-a-time is simple in its approach. However, since the effects of all factors are not considered at a time, it cannot assess the region of optimal response. On the contrary, statistically determined experiments are more precise than the classical

Published online: 16 March 2017