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Effect of chemical mutagens on expression of therapeutic protein-streptokinase in wild strain *Streptococcus equinus* VIT_VB2

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Streptokinase breaks down the clot in myocardial infarction, affecting three million people globally. The current study, enhanced the production of industrially important fibrinolytic enzyme, streptokinase (SK), this can be used to reduce death rate due to myocardial infarction. The ultra-violet (UV) mutated strain, UVSE6 of *S. equinus* VIT_VB2 showed maximum substrate specific-SK activity (864±0.6 IU mL^{-1}) and partial clot lysis (79%). Hence, the mutant strain UVSE6 was further enhanced by chemical mutagenesis. The improved mutant strain EMS1 after chemical mutagenesis showed maximum SK activity (1004.5±0.7 IU mL^{-1}) and partial clot lysis activity (89%), significantly higher than wild strain. The amidolytic activity of purified SK from mutant strain EMS1 of *S. equinus* VIT_VB2 was found to be 8253 ± 1.6 IU. The molecular weight of SK was determined as 47 kDa by SDS-PAGE and purity of SK was confirmed by HPLC (retention time: 2.82 min). Presence of *SK* gene isolated from EMS1 mutant strain *S. equinus* VIT_VB2 was confirmed using molecular gene sequencing (1200 bp). Structural analysis reveals 3.7% of the amino acid residues in outlier region in the wild type model increase in the mutant. The variation of amino acid in the sequences is observed in RAMPAGE analysis.

Keywords: Amidolytic activity, Clot busters, Ethyl methyl sulphonate (EMS), N-methyl- N′-nitro- N-nitroso guanidine (NTG), RAMPAGE analysis