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Screening, Cloning and Expression of Active Streptokinase from an Iranian Isolate of *S.equisimilis* Group C in *E. coli*

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ABSTRACT

Introduction: Streptokinase (SK) is a fibrinolytic protein secreted by β-hemolytic streptococci (βHS) groups A, C and G. Due to its importance as a thrombolytic drug, national screening programs in different countries for isolation of βHS and especially SK-producing group C (GCS) strains have been conducted. Herein, we provide data of the first screening study on βHS isolates in Iran for the aim of recombinant SK (rSK) production from a local strain.

Materials and methods: 252 streptococcal samples were collected and characterized using microbial/biochemical assays. The GCS strains were serologically confirmed. Activity of GCS supernatant cultures was determined by caseinolytic assay in comparison with the standard strain GCS9542. The SK gene of the highest producer strain was selected for production of rSK in *E.coli* system. The rSKs activities were determined using chromogenic assay.

Results: βHS were detected in 75 of the collected specimens (29.4%) including groups A (25.8%), C (3.6%) and G (0.4%). Analyses by SDS-PAGE and Western blotting indicated the proper expression of 47 kDa rSK proteins in *E. coli* for SK genes which were cloned from both the selected (GCS87-) and standard (GCS9542-) strains with the yields of 0.53 and 0.59 mg/ml (of the purified protein), respectively. The calculated activity for rSK 87 was around 90% of rSK9542 activity (0.18x105 IU/mg v/s 0.21x105 IU/mg).

Conclusion: Results of the present study for the first time provided the possibility of producing rSK from a local and native source with comparable yields and activities similar to the standard strain.

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Introduction

Pathologic blood clots (in the form of thrombus) can result in vascular blockage which can induce serious consequences including death (1). In a healthy haemostatic system, formation of blood clots is suppressed through conversion of zymogen plasminogen (Plg) to plasmin (the serine protease that degrades fibrin) (2). However, in pathological conditions, clinical intervention through application of plasminogen activators (also known as "thrombolytic or fibrionolytic agents") to relieve the vein from thrombosis is required. Currently, routine thrombolytic agents in clinical applications are recombinant

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