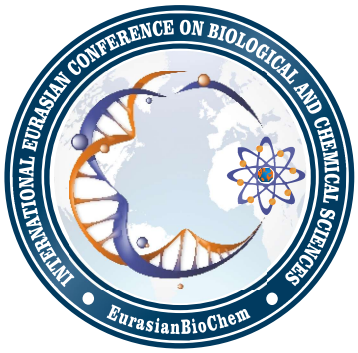

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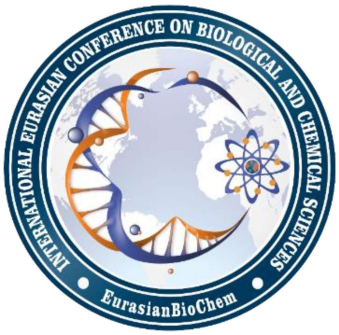

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ORAL PRESENTATION

Mini-prep protocol for simplified RNase A extraction

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Abstract

The enzymes produced by biotechnological procedures present variation in terms of the need for them in laboratory. Enzymes like DNA-directed DNA polymerase could be accepted as highly needed and frequently used enzyme in molecular biology laboratories. It is easy to find out DNA-directed DNA polymerase with the range of different cost and quality. However, there is limited options available for some other enzymes like RNase. In this study, it was aimed to extract RNase A enzyme by ethanol precipitation method by short time cost-effectively. First, expression cassette of the pOpenTaq plasmid was amplified by inverse PCR. Then, *RNase A* gene from pOpen-RNAseA was amplified by conventional PCR. Expression cassette and *RNase A* gene were ligated as a circulated vector, and then transformed into *Escherichia coli* JM107 strain. Confirmed colonies were used in RNase A extraction. Transformants grown in ampicillin (100µg/mL) added medium overnight, then they were induced with IPTG (1 mM) overnight. After extraction step, purification was carried out by 70% ethanol precipitation. In SDS-PAGE assays, 16 kDa protein was checked, and concentration was calculated by BCA protocol. The extracted RNase A (1:10, V:V) was used in SDS-based DNA extraction from 7-day-old mycelium culture of *Fusarium culmorum*. No RNA was detected in gDNA samples of RNase A-treated ones. The findings showed that the ethanol precipitation method could be easily used in any molecular biology laboratories involving common equipments to obtain RNase A enzyme within in short time.

Keywords: Enzyme extraction, molecular biology, PCR, RNase A

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