OPTIMIZATION OF DNA ISOLATION AND PCR PROTOCOL FOR RAPD ANALYSIS FROM MUCOPOLYSACCHARIDE RICH FOOT MUSCLES OF *LYMNAEA ACUMINATA* (GASTROPODA: PULMONATA) FROM INDIAN HIMALAYAN REGIONS

POONAM, N K TRIPATHI, PREETPAL KAUR & ANITA KUMARI

Department of Zoology, University of Jammu, Jammu, J&K, India

ABSTRACT

Genetic analysis of molluscs relies on high yields of pure DNA samples. Here we present the optimization of DNA isolation and PCR conditions for RAPD analysis of *Lymnaea acuminata* from Indian Himalayan region containing high levels of polysaccharides, polyphenols and secondary metabolites. Phenol-Chloroform involves Cell lysis in lysis buffer supplemented with Proteinase-K and KCl & three emulsified-washings with phenol: chloroform: isoamyl alchohol followed by precipitation with Isopropanol & overnight RNase treatment with all steps carried out at room temperature efficiently removed high protein and polysaccharide contamination. The yield of DNA ranged from 1000±1.63 to 973.08±0.88µg/ml and the purity (ratio) was between 1.821±0.002 to 1.856±0.001 indicating minimal levels of contaminating metabolites. The isolated DNA was used for randomly amplified polymorphic DNA (RAPD) analysis. RAPD protocol was optimized based on the use of higher concentration of $MgCl_2$ (2.5µl), lower concentrations of *Taq* polymerase (1µl), 1.0µl of template DNA and an annealing temperature of 35°C & 37°C, resulted optimal amplification. Reproducible amplifiable products were observed in all PCR reactions. Thus, the results indicate that the optimized Phenol-Chloroform protocol for DNA isolation and PCR was amenable to molluscan species which is suitable for further work on diversity analysis.

KEYWORDS: Phenol-Chloroform, PCR Amplification, Spectrophotometry, $Mgcl_2$, *Taq* Polymerase