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Further purification and determination of molecular masses of deoxyribonucleases from *Nepenthes distillatoria* and effect of metal ions on their activities

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The Pitcher plant *Nepenthes distillatoria,* is endemic to Sri Lanka. Very recently, we identified the presence of four deoxyribonucleases (DNAase) in the crude pitcher juice of *N. distillatoria.* Partial purification of DNAases present in the crude juice was performed using anion exchange chromatography followed by gel filtration chromatography at 4 ˚C. Stability of the enzymes at different temperatures and pH values were determined. The effect of metal ions on the DNAase activity was analyzed with the inclusion of test samples containing metal ions of interest (Zn^{2+}, Ca^{2+} and Mg^{2+}). Two major DNAases were further purified and their molecular masses were determined using SDS-PAGE and Gel filtration chromatography.
Results of the DEAE cellulose chromatography indicated the presence of four different DNAases (DNAase I, DNAase II, DNAase III and DNAase IV) in the crude pitcher juice. DNAases II and III were found to be the abundant enzymes. Optimum concentration of metal ions that enhanced activity in crude juice were, 4 mM (35% enhancement) and 6 mM (42% enhancement) for Ca^{2+} and Zn^{2+}, respectively. Activities of all four DNAases are positively enhanced by the presence of Zn^{2+}. Optimum concentration of Zn^{2+} that enhanced activity of further purified DNAase II and DNAase III being 4 mM (61 % enhancement) and 7 mM (90 % enhancement), respectively. A 1 mM concentration of Zn^{2+} enhances the activity of DNAase I by 30%, and that of DNAase IV by 49%. A 1 mM concentration of Ca^{2+} also show positive enhancement of activities of DNAase I (30 %), DNAase II (14 %) and DNAase III (13 %). A 1 mM Mg^{2+} does not seem to affect the activity of the four enzymes substantially. The molecular mass of purified DNase II was determined to be 52 kDa by Gel filtration and SDS – PAGE and that of DNAase III was determined to be about 34 kDa by gel filtration. Further, the reported thermal stability of all four enzymes over a broad pH range (pH 3.0 to 8.0) was confirmed.