CHARACTERIZATION OF DEOXYRIBONUCLEASES FROM PITCHER JUICE OF *Nepenthes distillatoria*

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Nepenthes distillatoria is a carnivorous plant endemic to Sri Lanka, which grows in South West and Southern parts of the island. Preliminary studies indicated the presence of deoxyribonucleolytic (DNAase) activity in the crude pitcher juice of *N. distillatoria.* This study was carried out to partially purify the DNAases and characterize them in terms of their optimum temperature, optimum pH, and stability at room temperature and 37 °C and the effects of metal ions on their activity and molecular masses.

First, the assay procedure was developed for analyzing DNAase activity in *N.distillatoria* using unpurified pitcher juice. This developed assay procedure was used throughout the study, to investigate the DNAase activity. In this study four major DNAases (DNAase I, DNAase II, DNAase III and DNAase IV) were partially purified from crude pitcher juice of *N.distillatoria.* Out of the four, two DNAases (DNAase II and DNAase III) were further purified and molecular masses of these were determined to be 51 kDa and 37 kDa for DNAase II and DNAase III respectively using gel filtration method. SDS PAGE of DNAase II revealed the molecular mass to be about 51 kDa.

DNAase II and DNAase III are stable at room temperature and 37 °C over a wide pH range (pH 3.0 – pH 8.0). However, more remaining activity is observed at acidic pHs. In all pHs DNAase II had over 50% activity remaining after 15 days of incubation at room temperature. At 37 °C after 15 days over 50% of activity of DNAase II remained at acidic pHs while it was less at pH 7.0 (46%) and 8.0 (26%). More than 59% of activity remained after 15 days at room temperature in all pHs for DNAase III. Interestingly the activity of DNAase III seem to diminish substantially at 37 °C.

The effect of metal ions on both crude juice and the partially purified DNAase activity were analyzed using the same assay procedure. Results indicated that, for crude juice the optimum concentration of Zn^{2+} was 6 mM and resulted 42% enhancement of activity. Optimum concentration of Ca^{2+} was found to be 4 mM and resulted in 35% enhancement of activity. However, the activity seem to be unaffected by the presence of Mg^{2+}. Activities of all four enzymes are positively enhanced by the presence of 1 mM of Zn^{2+}. (DNAase I - 40%, DNAase II – 28%, DNAase III – 24%, DNAase IV – 29%). 1 mM concentration of Ca^{2+} also show positive enhancement of DNAase I (30%), DNAase II (14%) and DNAase III (13%). Furthermore it was observed that optimum concentration of Zn^{2+} for DNAase III was 7 mM and resulted 90% enhancement of activity. For DNAase II optimum concentration of Zn^{2+} was 4 mM at which activity enhanced by 61%.

These results demonstrate the stability of the two major DNAases over a broad pH range both at room temperature and at 37 °C as well as their metal ion dependency. Their activity is enhanced by the presence of Zn^{2+} and Ca^{2+} ions. The difference of the separation on anion exchange column indicate that overall charge of these proteins differ. The differences of the molecular masses also indicates that these are different enzymes.

Furthermore, these enzymes seem to be resistant to the proteinase activity in the crude pitcher juice. Therefore, DNAases present in the pitcher fluid of *N. distillatoria* may have remarkable properties to withstand high temperatures, a wide pH range as well as attack by proteinases. These features clearly indicate the wide applicability of the enzymes.