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Immunohistochemical analysis of minor acid proteinase of *Nepenthes distillatoria*

Previous investigations conducted clearly showed the extraordinary properties of *Nepenthes* proteinases and it is clear that they are novel members of the aspartic proteinase family. The structure elucidations of the proteinases are very important to relate their properties to structure. Therefore, their exact tissue localization is essential for mRNA isolation and cDNA cloning. In this study immunohistochemical staining was performed on transverse sections of the pitcher of *Nepenthes* obtained under freezing conditions for the exact tissue localization of the minor acid proteinase.

Immunochemical staining of dot blotted *Nepenthes* minor acid proteinase with serum collected before primary innoculation and after 1st & 2nd booster, confirm that rabbits produced a specific antibody against minor acid proteinase. 1 : 4000 diluted serum was sufficient to detect 0.05 µg of *Nepenthes* minor proteinase. Immunochemical staining of blotted *Nepenthes* minor proteinase and other standard proteins with 1 :1000 diluted serum did not produce any visible band. This confirms that produced antibody does not cross react with other proteins and is suitable for histochemical staining. The antibody was purified by (NH4)2SO4 saturation and affinity chromatography on protein-A sepharose. The protein concentration of the purified IgG was 1.5 mg/mL. Immunochemical staining of the dot blotted minor proteinase suggested that 1 : 8000 diluted purified IgG preparation can be used to detect 0.01µg of the protein.

Fresh *Nepenthes* pitchers were divided into 4 parts (1 & 2 in the lower 1/3 glandular region and 3 & 4 in the upper 2/3 non glandular region). Transverse tissue sections of each part were subjected to immunochemical staining with 1 : 8000 diluted antibody preparation. A blue colour with alkaline phosphatase or brown colour with peroxidase substrate was observed only in the transverse sections of the lower 1/3 part of the pitcher. Microscopic examination of stained slides suggests that most of the parenchyma cells which are present as clusters and located in the inner surface of the pitcher have stained with antibody. This result demonstrates that the *Nepenthes* minor proteinase is synthesized in parenchyma cells located in the lower 1/3 part of the pitcher.