E2-02 Tissue localization of acid proteinases of *Nepenthes distillatoria* (Badura)

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Isolation, purification procedure and some properties of a major and a minor acid proteinase of *Nepenthes* juice were reported. Results clearly demonstrated their distinguishing properties, suggesting that they are unique members of the aspartic proteinase family. In this study, tissues which synthesize these 2 acid proteinases were investigated.

Procedure of polyacrylamide gel electrophoresis (with 12.5% gel and at pH 8.3) under non denaturing conditions followed by activity staining was developed to identify and separate the major and minor acid proteinases by using purified enzyme preparations. (To visualize proteinase, the gel was dipped in 2.0% haemoglobin at pH 3.0 and incubated at 37°C for 1h). The major acid proteinases migrate faster with the dye front and appeared as broad white band at the bottom of gel. A minor acid proteinase had slower migration and appeared as narrow band at the middle of the gel. Tissue localization of 2 proteinases were studied by analysing the crude extract of different regions of the pitches by using developed activity staining.

Pitcher was divided into 2 main regions as glandular region (bottom 1/3) and non glandular region (upper 2/3) according to light microscopic examination. There are a large number of secretary cell clusters present in the glandular region. Each cell cluster consists of a central pore surrounded by longitudinal secretary cells and parenchyma cells. Most of these cell clusters are gathered at the regional innermost layer of the pitcher wall.

Crude extracts of the lower 1/3 of the pitcher clearly showed 2 bands at the middle and bottom of the gel and no activity was detected with upper 2/3, after electrophoresis and activity staining.

These results suggest the presence of both (major and minor) acid proteinases in the cells of lower region of the pitcher. This finding and the anatomical structure of the pitcher suggest the higher probability of synthesizing these proteinases by the secretary cells and secreting into the juice.

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