618/E2

Preliminary characterization of phosphatase activity in the crude pitcher fluid of *Nepenthes distillatoria* (bandura)

Dilini Jinasena[1], Sanath Rajapakse[1], Preminda Samaraweera[1] and Senarath B P Athauda[2*]
[1]*Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya*
[2]*Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Peradeniya*

Carnivorous pitcher plants of the genus *Nepenthes* acquire substantial amounts of nutrients from the insects captured. A mixture of hydrolytic enzymes is believed to be responsible for efficient digestion of insect carcasses inside the pitcher fluid. In this study we partially characterized phosphatase activity detected in the crude pitcher fluid of *N. distillatoria,* a plant species endemic to Sri Lanka.

A simple assay procedure was developed using *P*- nitrophenyl phosphate (pNPP) as the substrate to determine the acid phosphatase activity in the crude pitcher juice. The reaction mixture contained 100 µl 0.5 mM pNPP, 100 µl 0.5 M sodium acetate buffer at pH 4.0, and 50 µl of crude *Nepenthes* fluid. After incubation at 37°C for 2.5 hours, 1 ml of 0.5 M NaOH was added to inhibit the reaction and color development and absorbance was measured at 405 nm. Optimum pH for phosphatase activity was determined by incubating the reaction mixture at different pHs ranging from pH 2.0 to 10.0. Optimum temperature was determined using the same assay procedure at pH 4.0 and at different temperatures from 30^0C to 60^0C. Thermal and pH stability of phosphatase activity was determined by incubating the crude pitcher fluid at different temperatures and pHs for a period of one month. Aliquots were removed at different time intervals and the percentage remaining activity of each aliquot was determined. Phosphatases in the crude pitcher fluid were partially purified using a DEAE cellulose column.

A significant phosphatase activity in the crude pitcher fluid was observed. Two pH optima were observed at pH 2.5 and pH 4.0. Optimum temperature for phosphatase activity was 55°C. The phosphatase/s in crude juice is not thermally stable as acid proteinases reported previously. More than 50% of activity was lost after 14 days at room temperature and within a day at 50°C. At 37°C and 45°C, 50% activity was lost within 6 and 3 days, respectively. However the enzyme was highly stable at low pH. More than 95% of activity was remaining after a month at pH 2.0, 4.0, 5.0, and 6.0. At pH as high as 9.0 and 10.0, approximately 50% of enzyme activity was retained even after a month. DEAE cellulose chromatography resulted in two sharp peaks at NaCl concentrations 0.10 M and 0.18 M, indicating the presence of two phosphatases in the crude juice. Studies on purification and characterization of these enzymes are in progress.

*sbpa@pdn.ac.lk Tel: 081-2396327