Characterisation of lichenase isozymes from the herbivorous gecarcinid land crab, *Gecarcoidea natalis*.

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The Christmas Island red crab, *Gecarcoidea natalis* consumes leaf litter and is able to digest substantial amounts of cellulose and hemicellulose using its endogenous cellulase and hemicellulase enzymes. While the cellulase enzymes have been characterized, the hemicellulases have not. In this study, enzymes with lichenase activity, that is the ability to hydrolyse the hemicellulose lichenan, were purified and characterized from the midgut gland of *G. natalis*. Three isozymes, termed 1a, 1b and 2, with molecular masses of 53, 43 and 47 kDa were purified to homogeneity. Surprisingly, these enzymes also possessed endo-β-1,4-glucanase (cellulase) activity and thus were re-classified as 1,4-β-D-glucan glucohydrolases to reflect their general hydrolytic ability. The enzymes hydrolysed internal glycosidic bonds to release short oligomers of 4-5 glucose units in length. These oligomers could be hydrolysed further; cellotetraose was hydrolysed into cellobiose; cellotriose was hydrolysed into cellobiose and glucose. Cellobiose could not be hydrolysed and thus the isozymes did not possess β-1,4-glucosidase activity. Using zymography, the three isozymes were identified in the digestive juice of *G. natalis*. They were also present in the digestive juice of other land crab species such as the closely related gecarcinid, *Discoplax hirtipes* and the anomurans, *Coenobita perlatus* and *Birgus latro*.