Chapter 57

Chitinase Assay by Spectrophotometric Method

Abstract

The chitin degrading enzyme chitinase is the most abundant polymer next to cellulose. Chitin is present in the cell wall of fungi. Chitinase hydrolyzes β-1,4 glycosidic bonds between the N-acteyl glucosamine residue of chitin. Certain setbacks with the existing chitinase assay method are either time bounding or less sensitivity in the assay methods. Spectrophotometric method is an important method in the assay of chitinase.

Keywords Chitinase assay, Spectrophotometer, Fungi, Hydrolysis

1 Materials

1. Colloidal chitin.
2. DNS reagent.

2 Methods [1]

1. For the chitinase assay, take the colloidal chitin as a substrate and add with partially purified/purified chitinase enzyme.
2. Add 0.9 ml of 1% (w/v) colloidal chitin with 0.1 ml of crude enzyme, incubate at 55 °C for 1 h.
3. By the addition of 3 ml of DNS, stop the reaction then heat at 100 °C for 5 min.
4. Centrifuge it, estimate the reducing sugar by DNS method.
5. Dilute the sample, measure the absorption at 530 nm using UV spectrophotometer along with the substrate and enzyme blanks.

Reference

1. Vahed M, Motalebi E, Rigi G, Noghabi KA, Soudi MR, Sedeghi M, Ahmadian G (2013) Improving the chitinolytic activity of *Bacillus pumilus* SG 2 by random mutagenesis. J Microbiol Biotechnol 23(11):1519–1528

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