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Kinetics Properties of Marine Chitinase from Novel Red Sea Strain of Bacillus

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Abstract

An aerobic Gram variable rod shaped polarly flagellated marine bacterium abbreviated R2 attracted our attention not by its hyper chitinolytic activity but also by its multiple enzymes production (protease, gelatinase, lipase, amylase, dextranase,alginase, arabinase, agarase, etc.). This bacterial isolate was selected and identified using conventional methods as well as 16S rRNA technique and submitted in the Gen Bank sequence database as Bacillus sp. R2 with a given accession number DQ 923161. Its purified chitinase showed a molecular weight of 41.68 KDa. Kinetics study revealed that the chitinase exhibited Km, Vmax and Kcat values of 6.971 mg/ml, 69.63 U/ml and 1.815 µcatal for colloidal chitin, 3.334 mg/ml, 83.32 U/ml and 2.172 µcatal for squid chitin respectively. Furthermore the enzyme catalytic efficiency ($Kcat/K_m$) was estimated to be 0.260 and 0.651 µM $P.min^{-1}mol^{-1}mg^{-1}S$ toward colloidal chitin and squid chitin, respectively. This is the first contribution about marine chitinase kinetics study from novel gram variable Bacillus isolated from the Red sea.

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Keywords: Bacillus sp. R2; 16S rRNA identification; chitinase; purification; kinetics.

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1. Introduction

Chitin, a β- (1-4) homopolymer of N-acetyl - D- glucosamine (GlcNAc), is the second most abundant polysaccharide existing in nature after cellulose. Chitinases (E.C.3.2.1.14), which are distributed in variety of organisms in the biological world, are a glycosyl hydrolases that act on the β-(1,4) linkage of chitin polymer. [1] During the last decade, chitinases have received an increased attention due to their wider ranges of biotechnological applications especially in the biocontrol of fungal phytopathogens [2] and harmful insects [3]. They have also been used as vaccine [4] as well as in the preparation of pharmaceutically important chitooligosaccharides [5,6].

It was well known that the substrate type and concentration affect the chitinase activity and subsequently the k_m and Vmax values. The numerical value of the Michaelis constant (K_m) is of interest for some reasons, among then knowing the Km value can help to adjust the assay condition so that the [S] > Km and there by determine the V-max moreover give an idea about the apparent affinity for the enzyme to its substrate. (The best substrate is that which has the highest Vmax/Km ratio). A wide range of bacterial chitinases particularly were, purified, and characterized7, 8, 9, 10] however very little reports or papers described their kinetics, for this reason the present work was a detailed study which has been carried out with the aim to determine all the kinetics parameters of Bacillus sp. R2 chitinase which was previously produced, purified, and characterized [11,12,13,14].

2. Materials and methods

2.1. Substrates and chemicals

Chitin was extracted from crustaceans and squid by the method of "Synowiecki*et al*. 1982 [15]", Crab shell chitin flakes (Win-lab, UK).Swollen chitin was prepared according to "Monreal and Reese, (1969) [16]"Peptone ,tryptone, and yeast extract were obtained from (Oxöid Hampshire, England). N-acetyl glucosamine, and bovine serum albumin (BSA) were from (Sigma -USA), 2 Hydroxy 3,5 dinitrosalselic acid (DNSA) obtained from (Merck, Darmstadt- Germany). All other chemicals and reagents that were used were of highest grade commercially available.

2.2. Microorganism and purified chitinase assay

*Bacillus sp.*R2marine bacterial strain isolated and identified biochemically and molecularly by cheba et al 2006 (strain accession number in NCBI GenBank was: DQ923161). The chitinase enzyme was produced , purified to homogeneity[11,12] and characterizedas reported in[13,14]. Chitinase activity was analyzed according to the method of "Miller (1959) [17]" by estimating the released reducing sugars spectrophotometrically at 540nm. A standard curve was established prepared with a series of dilutions of N-acetyl – D-glucosamine (NAG) and DNSA.One unit of chitinase activity was defined as the amount of enzyme required to release 1 μ mol of NAG per minute during reaction conditions.

2.2.1. Effect of enzyme concentration on chitinase activity

Different volumes (25, 50, 75, 100 and 200 μl) of the purified chitinase were used in excess of substrate to determine the suitable amount of enzyme to be added to the reaction mixture. Reactions were conducted at pH 7.5 and 37 ◦C for 30 min then the enzyme activity was determined as before.

2.2.2. Effect of reaction incubation time on chitinase activity

Reaction mixtures were incubated at 37°C and pH: 7.5 for various time intervals (0.2, 10, 20, 30, 40 and 60 min) then the enzyme assay were completed and the activity was measured as above.

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